

TRANSLATIONAL RESEARCH

Pioneering Research in Cryoscience and Cryomedicine: From Fundamental Investigations to Clinical Implementations. Theoretical, Experimental & Practical Experience. Scientific Facts 1979 - at present

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The key scientific events of the pioneering theoretical, experimental and clinical research and its implications in practice, which for the first time were carried out, consist of:

- For the first time ever, in the 1980s, the definition of modern cryosurgery was introduced as well as the fundamental theoretical, experimental, and clinical studies began on modern cryosurgery and cryotechnology in collaboration with engineers and designers.
- A number of theoretical and experimental studies, both *in vitro* and *in vivo*, have been performed to explain the action of low temperatures on tissue. The basic theoretical aspects due to the own theoretical studies are:
 - new experimental model was developed for the investigation of the dynamic temperature field of the frozen zone and the definition of the four phases for the effect of low temperatures on living tissue
 - investigations of the effect of freeze-thawing processes using temperatures of varying intensity from -40 °C to -180 °C by means of disc-shaped cryoprobes with diameters of 5 to 50 mm respectively, and a laparoscopic needle with a diameter of 10 mm *in vitro*
- The scientific implication of the longstanding research clearly is to define that the early and late cryosurgical vascular changes and circulatory stagnation together with the following cryoaponecrosis and cryoapoptosis as well as cryosurgical anti-angiogenesis and blocked the tumor vascularisation with immediately stopping oxygen supply to the tumor cells are the main important mechanisms of living liver tissue damage as a response to the freezing-thawing process. This initiated an irreversible damage tissue process and finally leads to complete destroying of tumor mass. These are the main mechanisms of biological living tissue injury following the low temperature exposure. Especially these mechanisms are well elucidated and illustrated at the deep temperature of -180 °C.
- The several experimental studies of the biological living substance in the animal experiment provide a platform to profoundly understand the mechanisms of damage and the pathogenesis of frostbite during low temperature exposure. The cryosurgical response of parenchymal tissue, i.e. ultrastructural cellular changes in parenchymal organs (liver and pancreas tissue), were investigated. This gives rise to a new concept concerning the

technical requirements of cryosurgical equipment and modern cryosurgery to perform modern cryosurgical operations, especially in oncology.

- The findings of the investigations allow clearly defining the clinical implication for the main technical requirements to cryosurgical devices. The main technical parameter stipulating for the effective cryodestruction is the provision of a high freezing rate of the biological tissue followed by further deliberate thawing.
- The unique phenomena have been observed and discovered in living matter which have become fundamental discoveries of importance to all of medicine. The discoveries are especially valuable for all of cryomedicine and cryoscience. The pioneering contributions represent a breakthrough in medical science, especially in cryoscience, cryomedicine, cryosurgery, and cryotechnology. It has been carried out crucial achievements in the rapid development of applications of medical importance. It has been done essential steps in the evolution of modern cryoscience, cryomedicine, cryosurgery, and cryotechnology.
- The Phenomenon "*Cryogenic Protein Denaturation*": Here it was discovered a natural phenomenon and shown the early and late ultrastructural cellular outcomes in hepatocytes after low-temperature exposure *in vivo*, using transmission electron microscopy. Immediately and one hour after freeze-thawing, hepatocytes display a disrupted plasma membrane, and mitochondria are observed with electron-translucent matrix and disrupted membranes. Electron-dense inclusions of denatured protein are also notably present. Immediately and 24 hours after the cryogenic procedure, full lysis of plasma membranes and the presence of picnotic nuclei are observed. Mitochondria with lysed matrix and disrupted external and internal membranes are once again observed, as are the denatured protein bodies. He regards as most significant novel observation of intracellular cryodenatured protein inclusions. Such freezing-induced denatured protein bodies have not previously been reported *in vivo*.
- The formation of intracellular protein inclusions following freezing *in vivo* is a novel observation. This observation would be of great general interest and provide the scope for interdisciplinary research in the fields of space science, biophysics, biochemistry, biology, and engineering, as well as in cryoscience and cryomedicine, cryoengineering and space medicine. This is next of the main mechanisms of deciphering of low temperature impact on living matter in nature.
- However, in presented research, the nano-structures of the living substance were fragmented and denatured into smaller nano-particles following deep low temperature exposure, leading to the deep low temperature interitus of the living matter on a nanometric scale and, finally, to local irreversible and avital lethal cell cryogenic explosion, namely *cell cryo-non-vita*.
- The Phenomenon "*Lunar Eclipse*": The early and late ultrastructural cellular changes, which were presented by the exposure of low temperatures at -80 °C and -180 °C *in vitro*, refer to the fundamental pathogenetic mechanisms in living matter. The properties of the living biological structures response after deep low temperature exposure provide important insights into the mechanisms of damage and the cryogenic lesion after the freeze-thawing

process in its different phases in the field of cryoscience and cryomedicine, i.e. cryosurgery.

- The Phenomenon "*Anthill Provocation Stimulus-Phenomenon*" (APS-Phenomenon): There are natural phenomena that can be characterized by their analogy with physiological and pathological processes in the human organism. An anthill that constitutes an autonomous balanced subsystem can be transformed immediately into a "dynamic chaos" by exogenous interventions. Small impacts can already turn a snowfield into an avalanche through exponential dynamics. These images are using in order to demonstrate a dead end of surgical oncology resulting very frequently in fatal consequences and human tragedies. An external intervention ("operation") in an anthill will cause a defect which opens the previous "balanced state" of the entire closed ant autonomy locally the "gate" being created constitutes an anomaly to the system. This results in the destabilization of the entire system. A state of chaos being created – in this case temporarily only – will give impulses for activities by ants. The confused ants will accelerate their motion dynamics not only within the present system space, but expand it outwards as well.
- The Phenomenon „*Cryosurgical Avascular Tumor*" (CAT-Phenomenon): The carried out research results shown that when the cryosurgical freeze-thaw cycle is finished and the cryozones with the ice crater in the middle, and the ice margin with the line of demarcation are thawed, the healthy liver parenchyma has taken on the colour of the dye. This occurs after cryosurgical application on the multiple liver metastases, but the cryoextirpated liver metastases have not been dyed, the cryoextirpated liver metastases are not dyed as well. Only the healthy hepatic parenchyma is sensitive to colour, but large liver metastases with the post-cryosurgical zone are not sensitive to colour. The absence of dye indicates a post-cryosurgical avascular tumor area.
- The bacteriological findings were negative in all experimental studies ($P < 0.001$). The data generated by studies shown that the early and late ultrastructural pancreatic and hepatic cell changes seen in the postcryogenic zone after freeze-thawing *immediately, one hour and twenty-four hours* indicate the non-vital continuation of the whole post-cryosurgical destructive process, i.e. the phase of irreversible and aseptic cryocytonecrosis with a subsequent phase of irreversible aseptic cryoapoptosis. In all studied groups the bacteriological results have not yielded any growth of microorganisms ($P < 0.001$).
- The results of studies have shown that the early and late pancreatic and liver cell changes in the post-cryogenic zone being observed in the first *twenty-four hours* after the freeze-thawing cycles are the beginning phase in the whole post-cryogenic process. They lead to the next phase of aseptic cryoaponecrosis and then to aseptic cryoapoptosis which, in many weeks, will clinically finish by the formation of an avital post-cryosurgical crust and, finally, a post-cryogenic (post-cryosurgical) scar.
- The effect of deep low temperatures on living matter has been deciphered in cryoscience and cryomedicine. The early and late aseptic cell cryoaponecrosis with subsequent aseptic cryoapoptosis as well as cryosurgical anti-

angiogenesis are the basic important mechanisms of living biological systems damage as a response to the freezing-thawing process. The early and late ultrastructural changes in pancreas and liver tissue were especially pronounced at the deep temperature of -180 °C. The bacteriological results have not yielded any growth of microorganisms. Finally, the nano-structures of the living substance were fragmented and denaturated into smaller nano-particles following deep low temperature exposure, leading to the deep low temperature interitus of the living matter on a nano-metric scale.

- It has been proved that cold surgery is the first surgical technique to use anti-angiogenesis, especially in the treatment of malignant diseases.
- There were collected the pioneering clinical experience based on the own investigations in modern cryoscience and cryomedicine in the last 33 years due to the development and performance of thousands unique modern curative and palliative minimal interventional and organ maintaining cryosurgical operations in patients with several primary and secondary kind of malignancies: skin cancer and metastases, malignant melanoma, breast cancer and local recurrence, multiple large hepatic metastases, head pancreas adenocarcinoma, stomach and colon adenocarcinoma, rectal and anal cancer, thyroid tumor, tissue malignancy tumor, lymph node metastases, tongue and mouth floor tumors.
- The summarized a long-term follow-up clinical experience with cryosurgery for treatment of liver metastases. The data of this 10-year prospective, randomized clinical trial suggest that hepatic cryosurgery is effective in the treatment of resectable and non-resectable liver metastases.
- Novel modern cryosurgery was introduced not only in palliative minimal interventional oncology but in the anti-tumour treatment concept by patients with primary malignancies, especially breast cancer, malignant melanoma, thyroid tumor, stomach and colon adenocarcinoma, rectal and anal malignancies aimed at the prevention of local recurrence and distant metastases.
- Discoveries are changed current preoperative invasive malignant tumor diagnostics. Pioneer invasive cryodiagnostics were describe and introduced into the practice.
- Challenge in current invasive tumor diagnostics via invasive cryodiagnostics of malignant diseases in state of temporary primary tumor mass immobilization.
- New cryogenic techniques for malignant curative and palliative treatment in the field of dermatological abdominal, breast and thyroid cryosurgery, cryosurgery for soft tissue malignant tumors and lymph nodes metastases, etc. have been discovered and developed. The new concepts, discoveries, inventions and development of the unique high-tech and innovative cryogenic technology: instruments and devices are introduced in 51 National, European and International Patents.
- The original engineering developments have open up the novel era to translation the fundamental research to the clinical implications in the area of surgical oncology worldwide.

- The changes in the world science and medicine with all available scientific data of the own personal theoretical, experimental, clinical, and technological research experience in the period of time from 1979 until present have served as the objective scientific foundation to define the new terminology and describe the new definition in the field of using low temperatures, especially deep low temperatures. There are the following new terminologies and definitions: cryoscience, cryomedicine, cryogenic denaturation (cryodenaturation), cryogenic phenomenon (cryophenomenon), cryogenic apoptosis (cryoapoptosis), cryogenic necrosis (cryonecrosis), cryogenic anti-angiogenesis, nano-cryoscience, nano-cryomedicine, nano-cryotreatment, nano-cryoablation, nano-cryoengineering, nano-cryogenic technology, nano-cryoinstrument, nano-cryoprobe, nano-cryoneedle, nano-cryodevice, *etc.*
- The pioneering clinical experience combined with fundamental theoretical and experimental research has substantially contributed to establish a new discipline in science and medicine namely modern cryomedicine and cryoscience world-widely and will doubtlessly contribute to an upsurge in the use of this scientific and medical branch in the near future.
- On account of personal results achieved in the field of cryomedicine and cryoscience over the last three decades and based on fundamental theoretical, experimental and pioneering clinical and technological research, a new standard for surgical oncology was formulated in the 21st century.
- The novel research in cryoscience, cryosurgery and cryomedicine as well as cryotechnology has now led to benefit in human medicine and science included the implementation in practice of the research results raised the level of medicine in human civilization, deciphering of the effect of deep low temperatures on living matter, challenge in current invasive malignant tumor diagnostics based on the development of a new standard – invasive cryodiagnostics, amplification of organ maintaining and gentle invasive interventions on the human organism, formulation and introduction of new standards in surgical oncology, contribution to establishing a new discipline in science and medicine – cryoscience and cryomedicine, contribution to developing an innovative era for modern cryotechnology and cryoequipment *etc.* aimed at the change paradigms in medicine, especially in oncology worldwide.
- Numerous workshops and seminars on modern cryoscience, cryomedicine, cryosurgery, and cryotechnology have been performed at the several medical institutions world-widely (Austria, Germany, Hungary, Poland, Ukraine, Russia, Jordan, Iraq, Vietnam, Moldova, China, Romania, Japan, *etc.*).
- Therefore, the fundamental investigations and distinguished contributions to use low temperatures in medicine bridge science with practice. This is the Pioneering Translational Research in Cryoscience and Cryomedicine.

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