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THE M. I. SYTENKO INSTITUTE OF BONE PATHOLOGY, NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE: SCIENTIFIC BACKGROUND

ABSTRACT

The M. I. Sytenko Institute of Bone Pathology affiliated with the National Academy of Medical Sciences of Ukraine is one of the leading research medical institutions in the country. During its half-a century history the Institute has successfully developed and applied the allo- and xeno-tissue transplantation technologies. Currently, in continuation of the achievements of the previous generations, the Institute activity is aimed at further elaboration and implementation of the demineralized and denaturalized allo- and xenobone material in medical practice; investigation of the stromal mesenchymal stem autocells; and study of the bone matrix proteins.

KEYWORDS: *transplantation biotechnologies; alloplasty; xenoplasty; stromal stem cells; tissue-engineering constructions*

The Institute is more than 100 years. In 1907, the Congress of Industrials of the Donbass founded the Medico-Mechanical Institute in Kharkiv to treat the workers-industry victims. In 1926, this institute was headed by Prof. Mikhailo Sytenko and it was at the 2nd Congress of the surgeons held in 1929 year that he proposed to set the orthopedics and traumatology into a separate speciality. His initiative was supported and in 1930 year this speciality was singled out of surgery as a separate discipline [1].

In 1963, Prof. Oleksiy Korzh was appointed Director of the Institute. Many prominent scientists had worked under his leadership, among them Rashid Talishevskiy, Yuriy Delevskiy and others.

In his doctoral dissertation, entitled "Homoplastic of the large bone and joint defects", Prof. R. R. Talishevskiy laid the main clinical principles of bone transplant handling, emphasizing the rules for sterile obtaining, storage and processing of the anatomic material, making post-mortem diagnosis, critical donor's age and indications for transplantation of auto- and homo-tissue (allo-) grafts.

The Institute researchers defined the bone plastic zones: diaphysis, articular zone and joint proper. They were first who described the barrier function of joint cartilage in the onset of infectious complications during transplantation of homo- (allo-) grafts. These works laid the basis for many national and foreign clinic-experimental investigations in the field of homo- (allo-) plastic of the skeleton bones [3,4].

In 1962, the Department of tissue storage and transplantation was opened at the Institute which was headed by Prof. Yuriy Delevskiy. His doctoral dissertation, entitled, "Role of the isoimmune antibodies during homo- (allo-plasty) skin transplantations" provided the basis for development of national tissue transplantology. Prof. Delevskiy is also well-known for his works devoted to bone graft enrichment with medications and microelements as well as AB0 of antigen differentiation of human blood cells and tissues.

He first discerned the two types of antigen markers and described the ANAP phenomenon (agglutination-negative-absorption-positive) and CYNAP (cytotoxic-negative-absorption-positive) identifying latent antigen determinants of human tissues and cells. Almost half a century keeping ahead, these works allowed many followers avoid mistakes and found the major principles of immunological selection of donor and recipient tissue for transplantation [5-7].

At present, the Institute is the academic scientific-practical Centre of the country in the field of orthopedics and traumatology. Through its history, the priority research directions were: treatment of skeleton and joint diseases; and functional treatment of fractures and hemoplasty, the term used then for bone tissue allotransplantation [2].

The time has passed. At the 90es of the 20th century no further progress in transplantology was possible. At the stage of Ukraine transformation into an independent state, tissue transplantology was

annulled. After an almost 15-year silence in the year 2009 on the initiative of the director of the Institute Prof. M. Korzh a Department of bone tissue transplantology was opened.

The Department researchers familiarized themselves with the available works in the field of bone implantation and tissue transplantation as well as with the methods of bone implant construction from allogenic bones. Modern approaches to bone material reconstruction are based on the use of physical (ultrasound) and (chemical) factors such as aggressive agents: acetone and sodium hydroxide factors. As a result, biological properties of the bone tissue are altered under so aggressive influences.

The Department has developed an original methodology of extraction of non-collagenic antigen proteins by using softer and sparing ways allowing considerably increase medicine biocompatibility and almost completely reduce encapsulation and rejection.

As a result of using this new methodology, the bone becomes partly deproteinized and cleaned from the remaining non-collagenic antigen proteins capable to call immune response. Importantly, the collagen microstructure and mineral bone matrix remain undamaged what is required for preservation of osteo-inductive and osteo-conductive properties of the transplant.

The Department carries on numerous experimental investigations to study antibacterial effects of various chemicals and regimens of chemical bone material sterilization in the mammalians and to study antibacterial bone tissue acquired effects after saturation with various antibiotics and ascorbic acid. The given technique of intensive bone tissue saturation with the antibiotic, Ceftriaxone, allows, firstly, perform non-sterile uptake of the biomaterial and have no fear of secondary contamination during above-described processing, and, secondarily, reduce considerably the percentage of suppurations during its clinical use. The protocol consists of bone tissue saturation with antibiotics with addition of dimethyl sulfoxide for chemical enhancement of diffusion and constant mixing to physically accelerate the saturation process. At the final stage of an entire technological process the bone tissue is enriched with ascorbic acid under sterile conditions using electrophoresis.

At bone humidity above 6-8%, the temperature for transplant storage should be below zero. Storage term is 3 months. Such transplants now make 90% of the total processed material. At bone humidity less 6-8%, the bone material vacuum-packed into sterile cover can be kept at room temperature during 6 months. In recent years the given methodology has been mastered and applied to carry out thermostatic bone tissue dehydration and following “dry” sterilization by UV or radiation.

New technologies have been used in the Department during temperature bone tissue dehydration with preservation of native collagen structure and obtaining of bone micro granules to fill-in bone vacuum. Demineralized bone complex with elastic matrix is made and used **(Fig. 1)**.

New complex methodology for obtaining bioimplants from the xeno-bone or donor allo-bone has been created for the plastic of complicated human skeleton bone defects. This methodology includes physical, chemical and enzymic processing of bone material to ensure complete elimination of infectious agents (micro organisms, viruses, etc.) and autoimmune factors.

Further saturation of bone implants with vitamins, biologically active substances and medical preparations allows create agents of purposed-designed therapeutic action.

Method for bone tissue dehydration in special device has been elaborated allowing prevent thermal denaturation of the collagen in producing bio-available material. Owing to this method bone tissue can be split by means of the bone crusher.

A perspective direction has been proposed in clinical use of the obtained medical preparation in surgical treatment of the fractures which do not grow together, artificial joints and other disturbances of bone regeneration. In clinic, the bone micro granules are used separately or in the mixture with thrombocyte-enriched plasma, stem cells and other biologically active agents **(Fig. 2)**.

Figure 1. Demineralized bone elastic matrix.

Figure 2. Trabecular bone chips.

New methodology for bone tissue demineralization with the use of acids and more sparing neutral agents has been first developed in Ukraine. Experimentally, the optimal concentrations and decalcination modes were chosen. Demineralized bone matrix (DBM) is made from both, cortical and spongy bone tissue, depending on necessary properties of the preparation. Using the DBM, the plastic can be performed on especially complex defects requiring high plastic properties of the material. Besides the DBM has a considerable osteo-inductive potential.

Owing to modern technologies for isolation, identification and culturing of human stem cells, cell biotechnologies clinical are now used in the treatment of earlier incurable diseases. With special inductors it became possible to manufacture combined three-component tissue-engineering constructions on the basis of human stem cells which are differentiated into osteogenic, chondrogenic or neuronal directions. This makes treatment of loco-motor apparatus diseases more effective and qualified.

Modern methodologies of platelet-rich plasma activation by patient’s autothrombin and other bio-substances allow gain maximal concentration of growth factors in the preparation and in the required proportion depending on the task confronting the doctor. Thus the artificially formed complex of growth factors accelerates the regenerative processes without a considerable loss of organism local biological reserves.

Use of the materials manufactured on the basis of allo-bone is one of the perspective directions in the development of surgical orthopedics and traumatology under present conditions of development of bone plastic organ-preserving operations on the loco-motor system.

In a number of cases it is possible to preserve the limb and to recover its foothold, and the allo-bone is void of the drawbacks which are found in its “competitors”: metal and ceramic constructions.

Another perspective direction of the Department's research activity is the autotransplantation of mesenchymal stromal cells (MSCs).

The unique specificities of MSCs make them perspective for the clinical use:

- comparative easiness in obtaining from bone and tissues;
- a wide spectrum of possible differentiations into various tissues;
- limited proliferative potential allowing reduce the risk of tumor formation contrary to embryonal stem cells.

The MSCs possess immune-suppressive properties, a capacity to avoid immune system rejection and to inhibit lymphocyte proliferation in the response to the antigens. This makes them both acceptable for allogenic transplantation and used in suppression of 'graft versus host' reaction. Thus after having by allo- or xeno-bone transplant by bone marrow stroma auto-cells, we shall reduce considerably the risk of immune reaction and enhance osteoreparation.

Still, despite these wonderful properties and perspectives we should not close our eyes on the risk of malignant transformation of MSCs. For this it is necessary to perform in vitro cell culturing under control of the cytogenetic analysis as control of the biological safety of the bone marrow stromal stem cells. The stem cells thereby controlling genetic stability of cell lines and rejecting the strains with abnormal cells.

Finally, a perspective direction in bone-tissue system research is step-by-step combination of separate materials and methods into systemic clinical approaches: formation of allometal-composite transplants, their processing with platelet-rich plasma and MSCs, combination of tissue and cellular medical biotechnologies. A further development of this direction in orthopedics and traumatology assumes припускає the formation of створення зростаючих metal-allocomposite constructions for children pathology, vertebrology and for operations on the long bones of the locomotor system.

In the nearest time, we plan to produce high-quality individual allo- and xenografts for replacement of parts of the locomotor system at bone-plastic surgery.

Currently, along each of the perspective directions the Institute carries on experimental investigations and a thorough analysis of the obtained data with the use of prospect studies in biochemistry, immunology, biomechanics, etc. This allows substantiate the use of allo- and xenogeneic tissues in reconstructive surgery of human locomotor system.

Today, when resolving the problems of tissue and cell transplantology we stand on the shoulders of previous generations of the great scientists of the past who had made foundation for the coming generations.

REFERENCES

1. *Korzh MO.* Istorija instytutu. Spogady pro ljudej, doli, chas [History of the Institute. Memories of people, fate, time]. Harkiv: Prapor – Harkiv: Flag, 2007. 208 p.
2. *Goridova LD.,* Nasledie. Aleksej Aleksandrovich Korzh: nuchno-istor. izdanie [Heritage. Alexey Alexandrovich Korzh: scientific and historical edition]. Kharkov, 2014. 192 p.
3. *Korzh AA, Talishinski PP.* Gomoplastika v lechenii opuholej kostej. [The homoplasty in the treatment of bone tumors]. Kiev: «Zdorov'e» – Kiev: «Health», 1973. 105 p.
4. *Korzh AA, Moiseeva K.* Kostnaja zamestitel'naja alloplastika s pozicii otdalennyh rezul'tatov [Long-term results of bone replacement alloplasty]. Vestnik hirurgii – Bulletin of Surgery, 1981; **(11)**:94.
5. *Delevskij Ju P.* Jeksperimental'noe izuchenie specifichnosti i roli izoimmunnyh antitel, voznikajushhih pri gomoplasticheskih peresadkah kozhi: avtoreferat dissertacii na soisk. uchen. stepeni kand. med. Nauk Har'kov [Experimental study of the specificity and the role of isoimmune antibodies arising from gomoplastic skin transplantation: abstract thesis]. Kharkov, 1964. 16 p.
6. *Imamaliev AS.* Zagotovka i konservacija tkanej oporno-dvigatel'nogo apparata [Preparation and preservation of tissues of the musculoskeletal system]. Moskow, 1970. 224 p.
7. *Bondarenko NS.* Nekotorye biojelektricheskie pokazateli gomokozhi, konservirovannoj zamorazhivaniem pri razlichnyh ishodnyh uslovijah: dissertacija na soisk. uchen. stepeni kand. med. Nauk [Some bioelectrical indicators of homoskin which was preserved by freezing at different initial conditions: abstract thesis]. Kharkov, 1967. 266 p.

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