How to Cite:
Kovalev, A. V. (2022). Problems of creating bioartificial organs and the competition of medical technologies. *International Journal of Health Sciences*, *6*(S7), 3166–3209.
https://doi.org/10.53730/ijhs.v6nS7.12120

Problems of creating bioartificial organs and the competition of medical technologies

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***Abstract*---**A crucial direction in the development of biotechnology is production of human bioartificial organs and of related products for tissue and organ engineering – constituent components for biofabrications of organs and tissues: human biopolymers of extracellular matrix (ECM), cytokines, biomass of autologous and allogeneic human cells, organoids and tissue spheroids, as well as special equipment – tissue bioreactors, bioprinters, etc. The biotechnology development level today allows creating live _spare parts' for humans, and there is the prospect of competition with manufacturers of prostheses and implants. The present review considers the unsolved problems of creating bioartificial organs from the perspective of the classical triad of tissue engineering technology – cells, scaffold, and bioreactor. The author focuses on clinical examples and discusses possible strategies and new approaches for growing bioartificial organs inside *in vivo* bioreactors (hereinafter – regeneratrons) integrated with a living organism. Regeneratrons are the latest generation of tissue bioreactorsrarely used in clinical practice yet with a potential of increasing the efficiency of treatment.

***Keywords*---**Bioartificial organ, regenerative medicine, tissue engineering, regeneration, bioreactor, human trachea, transplantation, regeneratron, extracellular matrix; scaffolds.

Introduction

The review summarizes information on the scientific background, current progress and future potential of bioartificial organs, discusses the crucial studies on the problem and focuses on the main factors that impede effective cultivation of bioartificial organs. The causes of failures with the human bioartificial trachea are discussed in more detail, and special attention is drawn to the competition of tissue engineering approaches with innovative surgical

technologies for organ restoration, and to the need to draw a dividing line between these technologies. The possibility of building tissue in place of the lost parts of organs in *in vivo* bioreactors integrated with living objects is discussed. The review lists the advantages that allow not only controlling and managing the local regeneration environment but also combining the tissue culture method with optimal conditions for regeneration into a single system. This is potentially useful for overcoming the known obstacles of organ biofabrication, of further developing new advanced biomedical technologies for growing bioartificial organs in *in vivo* bioreactors and of transferring the technology into clinical practice.

1. The doctrine of bio-artificial organs.

Organ or tissue transplantation is usually performed in exceptional cases when no other therapeutic measures can save the life of the potential recipient or restore their health. Global organ transplant shortages are a widely recognized public health problem, and the World Health Organization estimates that only 10% of the world's organ transplant needs are satisfied.

A new field of medicine – regenerative medicine – lays the scientific foundation for producing (in artificial conditions from living cells) new organs or parts of them capable of fully interacting with the recipient organism. Two main types of such bioartificial organs can be distinguished – organs and tissues suitable for transplantation, immunologically compatible with the recipient and able to function for a long time inside a living organism, and those created for maintaining the viability of a patient whose own similar organs are so pathologically altered that they are unable to maintain their functions, and without intervention this condition is deadly. These are bioartificial organs created in the form of medical devices connected to the patient‘s circulatory system. They are aimed at

taking on certain functions inherent to this organ, at providing the time needed for the regeneration of the pathologically altered organs or for waiting for transplantation of donor organs. Such organs are called bioartificial, to distinguish them from artificial prostheses and implants.

The view on what a bioartificial organ is are constantly evolving (32). In the English-language literature, the spelling of the word is not defined; two options are used: hyphenated and not hyphenated. The term _organ‘ is well established and defined quite accurately –a body part as an evolving complex of tissues combined by a common function, structural organization and development. The word _bioartificial‘ itself is derived from two roots – the Greek _bios‘ (life) and _artificial‘ (not natural, man-made to be like genuine or instead of genuine, that is, associated with the conscious activity of a human being). Since the cell is the basic and minimum necessary unit of the structure and vital activity of all tissues, organs and organisms, today the definition of the term _bioartificial organ‘ can be as follows:

A bioartificial organ is a complex man-made object – a biological construct equivalent in some way to a healthy organ or a certain part of it. The most significant and obligatory part of a bioartificial organ are living cells located on

supporting biocompatible polymers with extracellular amorphous substance, which allows ensuring the correct cell-cell and cell-matrix interactions, long-term preservation of organ-specific gene expression, cell differentiation and prolonged performance of the functions inherent in a given organ by the cells and by the entire construct.

The American scientific community considers Howard Green, the honorary professor of cell biology at Harvard Medical School (USA) to be the founding father of regenerative medicine. Back in 1970, he learned to grow epithelial layers from skin epidermal cells in a culture to heal burns.

In the 20s of the last century, the Russian surgeon Ilya Alekseevich Golyanitsky first proposed the term

_regenerative surgery‘. He found that in all his experiments on organ and tissue transplantation, the transplanted material causes reparative processes on the part of the receiving tissue bed. The graft is an inducer of reparative regeneration - it stimulates the regenerative process or provides the very possibility of recovery. Subsequently, the regeneration of tissues and organs by living or specially treated organs and tissues transplanted from donors received its own name in the Soviet literature –_transplantation regeneration‘. A transplant from another person or an own transplant, serving as a framework for the germination of new tissue of the recipient, was dubbed _allostatic‘. During transplant regeneration, the volume of tissue being restored is small, usually from 1 to 2 cm between the edges or from the edge of the damaged organ. Unfortunately, studies of this variant of regeneration in the USSR were not sufficient.

The term _tissue engineering‘ was introduced in 1985 by the bioengineer Yuan-Cheng Fung in a proposal to the National Science Foundation (NSF) to finance the Living Tissue Engineering Center at the University of California, San Diego. The term was used to describe the methods to manipulate tissues and organs in surgery. In 1987, the concept was formally introduced into medicine and defined as follows: —Tissue engineering is the application of the principles and methods of technological developments and biomedical technologies for a fundamental understanding of the structure-function relationship in normal and pathological tissues of mammals and the development of biological substitutes for restoration maintaining and improving their functions‖.

One of the first organs assembled from devitalized ECM and cells of the epidermis, endothelium and fibroblasts was skin. For instance, Daniel A. Medalie cultured keratinocytes on the papillary layer of acellular dermis to create an artificial analogue of full-layer skin. The studies mentioned fibroblasts, endothelial cells from the bottom of the recipient‘s wound, penetrating into the acellular dermis transplanted onto the wound bed (28). Moreover, the germination of the vessels of the recipient went in the direction of their natural course - the same as in normal skin. The thickness of this _neoderm‘ exceeded the thickness of the restored skin after the engraftment of a split skin graft taken from the patient‘s own skin, which is usually transplanted by combustiologists.
Joseph P. Vacanti and Robert S. Langer are recognized as pioneers in the field of

tissue engineering, the area that applies the principles of biology and engineering to develop functional substitutes for damaged tissue. In 1993, they offered their vision of the foundations and problems of this interdisciplinary field and tried to find their own solutions for creating bioartificial organs and for tissue repair (22). Two main components necessary for tissue creation were identified: a group of living cells and a mechanical framework. The cells provide the necessary components for tissue repair while the carrier biopolymer base (mechanical framework) acts as a stable platform giving long-term support to the three-dimensional organization of cells and extracellular substance. Cells play three main roles in the development of new tissue: they ensure the regeneration of the construct, maintain the shape of the scaffold for a long time, and in a living organism they function together with intercellular substance as a stable set united by a common structure. The key characteristic of using cells in reconstruction is their ability to respond and adapt to internal and external stimuli *in vivo*.

2. **Banks of tissues and organs, 'pale ghosts' of organs.**

Tissue Bank is an institution that collects and processes tissues of a human corpse for the purposes of medical research, training and transplantation of allogeneic biomaterials to stimulate regeneration. Such biobanks provide high-tech production for procurement, preservation and storage of biological tissues. A tissue bank can store tissues from dead human bodies under cryogenic conditions. The most commonly used in medicine are osteoplastic

materials, fragments of tendons, cartilage, and biological coatings for wounds. They do not carry living cells; moreover, they are often additionally decellularized. Obviously, preservation of connective tissue structures is most common; this is due to the fact that most of connective tissue is represented by a well-stored non-living matrix that preserves its shape, has biological activity and initiates a special method of regeneration upon implantation into a living organism – regeneration by an implanted scaffold or matrix which subsequently undergoes natural destruction and resorption but creates a temporary model (framework) which allows local tissues to carry out regeneration in this volume.
Head of Bone Research Laboratory, the University of California, orthopedic surgeon, explained how demineralized bone matrix causes new bone formation even in an unusual place, for example, in muscles after intramuscular implantation. A major discovery in 1965 was bone morphogenetic proteins (BMP), capable of inducing non-specialized cells of connective tissue to create bone.

An interesting example is the creation of human BioBanks of devitalized (without living cells) human donor trachea to restore airway patency. For processing, lyophilization was used (most proteins are not destroyed and can be stored for a long time with moderate cooling, about 0°C). Lyophilized proteins, when moistened, restore their original properties, so lyophilized tissues are widely used by doctors in reconstructive surgery. Notably, as of today there is no clear indication that the allograft must be alive. However, transplanted living organs, like their scaffolds without living cells, are often also called allografts, which creates some confusion; _cadaveric homograft' is a good term for such

transplanted biomaterials. The merit of creating human trachea biobanks belongs to Claus K. Herberhold, professor of the Department of Otolaryngology at the University of Bonn (Germany) where donor tracheas are processed and stored in sterile solutions; when processing the trachea, immunogenic proteins are lost. In 1979, Herberhold transplanted an artificial trachea to an adult, and later artificial donor tracheas were transplanted to hundreds of patients with stenosis of most of the trachea, and these tracheas more or less successfully completed their task to restore breathing and save the patients. A distinctive feature of Herberhold‘s technology is the special preparation of the trachea for transplantation. Within a day after the donor's death, the trachea is taken in clean but not sterile conditions, moreover, without the tracheal muscle and with the very minimum of soft tissues around the cartilaginous half rings of the trachea. The isolated cartilage of the trachea is immersed for 14 days in 500 ml of 4% formalin solution with the addition of sodium lactate, then transferred for 56 days in 500 ml of the solution containing 4 g / l of thimerosal dissolved in Eagle‘s medium. Then the trachea is stored in acetone (including for lipid extraction) for at least 10 days before transplantation into the human body.

In this form, the trachea homograft comes from the trachea tissue bank (homograft bank) to the clinic for transplantation. In the lumen of the homograph, stents (for example, Dumon stent) must be installed for a long period. Such tracheal allograft can be used within two years from the time it was prepared. Similar tracheal allograft banks are currently created in Miami (Florida, USA), London (England) and Bonn (Germany).

Before transplantation, the prepared allograft is thoroughly washed in an isotonic solution. Most importantly, special studies confirm that all the cells in the allograft die, and that in formalin, all the proteins of the major histocompatibility complex markers are lost; therefore, such tracheal allograft does not cause immune rejection.

Later, in 1996, the surgeons of the Great Ormond Street Hospital(GOSH) in London published the work "Reconstruction of the trachea in children using a cadaveric allograft (homograft) trachea". Martin Elliott, professor of pediatric cardio-thoracic surgery at University College, London, Head of the National Department of Traumatology for Children at GOSH, and colleagues described five complex operations for the restoration of trachea and subsequent observations of children aged 5 months to 8 years who suffered from congenital long narrowing of the trachea, not amenable to conservative treatment, and who were in a life-threatening condition. All the children had a cadaveric homograft prepared in a similar way as described above; the transplant was carried out exclusively for health reasons. Immediately after transplantation, a stent was installed in the lumen of the donor trachea for three months, maintaining the diameter of the lumen necessary for normal breathing. Notably, fibroblastic reaction of human tissues and organs to trauma takes exactly three months– during this period, blood vessels and young connective tissue actively grow around the transplanted matrix of the trachea, causing contraction of the surrounding soft tissues, and also partially grow inside the lumen of the respiratory tract (16).

The phase nature of the regeneration of connective tissue is also important. First, the newly formed connective tissue has a certain structure (granulation tissue), and then regeneration maturation, or remodeling, takes place – namely, reduction of newly formed vessels, and contraction and compaction of regenerate with collagen fibers to form scar tissue. Stents prevent deformation of the new airway and narrowing of the lumen of the new trachea, but deformation can affect neighboring organs. After three months, the scarring process is completed, and the formed structures have a relatively stable shape, so stents can be removed. In 4 out of 5 children, adequate ventilation was restored after this operation; the observation period for the operated children ranged from 9 to 16 months.

During surgery, most of the narrowed trachea was removed, but the back of the organ (the tracheal muscle(membrane)) was kept for the graft to be attached to it (the entire back wall was removed from the graft during preparation). In other words, 2/3 of the new trachea was cadaveric (the organ frame from the donor with cartilaginous half rings), and the back wall of the patient‘s own trachea remained, being covered with own mucous membrane with ciliated epithelium and having own good blood supply. The remaining ciliated epithelium

regenerated and _crawled‘onto the side and front walls of the cadaveric part of the trachea, which underwent germination of young connective tissue, rich in blood vessels, and this contributed to the restoration of a more or less complete mucosa. The surgical technique allowed restoring the blood circulation of the tracheal walls due to the regeneration of the children's own vessels in 4 out of 5 cases. All the children underwent regular bronchoscopy in the postoperative period to prevent suffocation, and the granulation tissue excessively growing in the lumen of the trachea (usually formed at some distance from the stent) was removed, as it could prevent breathing. Such operation allowed all the surviving children (4 out of 5) to do without internal expanders, tubes or tracheostomy (connected holes in the skin and trachea) through which children with similar problems often breathe. Three of these children breathed freely without medical support within 16, 14 and 9 months after the operation, and one child continued outpatient treatment at the time the article was written.

On October 28, 2009, French surgeons from Avicenne Hospital in Paris successfully transplanted an artificial bronchus after removing the patient‘s own bronchus with a cancerous tumor, saving the 78-year-old patient‘s life. Notably, in elderly patients, bronchus removal is very risky, often leading to death. The French-made artificial bronchus consisted of a cadaveric cryo-preserved aorta (made in the same way as for vascular surgery and stored in a tissue jar), reinforced with a special metal stent on the inside. A big plus of such cadaveric aorta is the absence of the need for taking immunosuppressants, which is contraindicated for cancer patients.

The results of the operation were excellent, since two and a half years after, the patient felt very well, and most importantly, no cancer recurrence developed over this period. ―Our approach is based on the previous ten years of animal research,‖ said lead researcher and thoracic and vascular surgeon Emmanuel Martinod, yet

he emphasized that it is still necessary to carry out such surgical interventions in humans with caution.

The choice of aorta was determined by the appropriate shape. According to the authors, this is the same tubular canal with a diameter comparable to the diameter of the trachea. In addition, the strength, elasticity and resistance of the aorta to infection are well known. On the other hand, elasticity is the main disadvantage of the aorta: there is a risk of collapse of its walls. However, this can be avoided by installing a special metal stent. In animal experiments, complications did not occur in most cases of aortic implantation, at least in the subsequent three years after surgery. In these experimental studies, partial regeneration of tracheal tissues after transplantation of cryo-preserved aortic allografts was described. Cartilage formation was documented, and the source of regeneration, according to the French authors, was the recipient's own mesenchymal bone marrow cells which usually circulate in the blood. In modern literature, they are often called circulating fibrocytes. Histological studies of regenerates in animals showed a gradual regeneration of the respiratory epithelium of the bronchi with metaplasia from squamous to mixed, and then to normal mucociliary epithelium. The convincing advantage of using cryo-preserved aortic allografts was shown in comparison with the decellularized trachea and with the aorta preserved in glutaraldehyde. The French scientists demonstrated that an aortic allograft can be a valuable substitute for an unusable bronchus, with a result similar to transplantation of a donor bronchus, but in this case no suppression of immunity is required (33).

The experimental work on bronchial regeneration was carried out by Martinod at the Laboratory of Biosurgery of cardiac surgeon, former President of the French Academy of Sciences Professor Alain FrédéricCarpentier, who received worldwide fame for the development of heart valve bioprosthetics. Professor Carpentier called this case _the magic of bronchial regeneration‘, explaining that the transplanted aorta became an extremely successful matrix for bronchial regeneration, since it was covered with a new ciliated epithelium that was able to clear the airways (41).

A new approach in tissue engineering for creating bioartificial organs is the technology of bioengineering whole organs, based on the decellularization of entire donor vital organs for populating exposed connective tissue fiber cages (scaffolds) and basal membranes of these organs with living recipient cells. Bioreactors of special designs are used that support the viability and proliferation of cells among the framework for its _revitalization‘ and possible engraftment in the recipient‘s body with further improvement of the technology. New scientific term

_whole organ tissue engineering‘ emerged, and the bioartificial organs thus obtained are called _ghost organs‘ or

_ghostly floating organs‘(4, 29, 30).
With this approach, an isolated and already dead donor organ is thoroughly perfused with special detergents to remove all cellular and immunogenic species while preserving the extracellular matrix (ECM) and potentially integrated

vascular network. In other words, decellularization is the process aimed at removing cells from tissue while preserving the ECM and the three-dimensional structure of the organ.

A large number of studies on creating bioartificial organs inside special devices – bioreactors (in other words, cultivation of organs or tissues *in vitro*) are carried out on the basis of a matrix of donor organs. The cultivation of new organs is carried out on the basis of the matrix, primarily the fiber skeleton of donor organs. All cells are washed out of the donor organs using special solutions, more precisely, special detergents. The following ingredients are most often used: 1) sodium salt of laurylsulfuric acid, anionic surfactant, cleaning and wetting agent (SDS);, 2) Triton X 100 (non-ionic surfactant); detergent for DNA extraction as part of a lytic buffer; 3) solution of calcium chloride and magnesium sulfate to activate endogenous nucleases; 4) dioxyribonuclease 1 - human endonuclease synthesized mainly in the tissues of the digestive tract (DNase I). This technology of preparing an

organ matrix for tissue engineering is called perfusion decellularization, sometimes a similar method is called detergent-enzymatic decellularization.

The remnants of the extracellular matrix, primarily the fibers that form the skeleton of the mammalian organs, behave as a support and sites for fixing cells; they probably regulate the maintenance of the differentiated phenotype of cells and more complete intercellular interactions.

Today, improvements in the phases of decellularization, recellularization and monitoring of performance of transplanted hearts allow approaching a viable and functional bioengineering equivalent.

These tissue engineering technologies potentially allow the use in clinical practice of donor organs that are rejected due to poor condition, are considered unsuitable for transplantation, but are suitable for generation of decellularized scaffolds for tissue engineering of whole organs, since living specialized cells are not needed in this case , and the fiber skeleton of any organ retains its properties outside the host organism for a sufficiently long time, and is less demanding on storage conditions.

Restoration of the vascular network is an unresolved problem of tissue engineering and regenerative medicine; however, among the various compartments stored in the decellularized organs, the extracellular matrix of the vascular network remains the most intact, retaining its architectonics for a long time, surrounding hollow channels, which allows the use of natural pathways for the introduction of cells more evenly throughout the body.

Descriptions of new approaches to organ decellularization, repopulation and maturation which are used to date and new whole organ tissue engineering technologies are of potential interest.

Most often, blood is transfused and bone is transplanted; vital organs are in demand for transplantation in the following sequence: kidney, liver, heart.

Kidney

The first attempt to engineer the entire kidney tissue was described by Ott's team. Their study described the decellularization of different types of kidneys, such as rat, pig and human. They were able to populate rat kidneys with HUVEC and kidney cells in newborn rats, achieving a spatial distribution that resembled native glomeruli. Dynamic cultivation of the seeded cells led to a drop in vascular resistance and a sign of vascular function. The group performed short-term orthotopic implantation of an artificial kidney by anastomosis of both renal pedicles. They reported a clear vasculature that avoided blood clots, although it was not clear how long the transplant remained in place (Song et al., 2013). Interestingly, they reported that the ECM matrix was able to spatially instruct the cells and direct them to the correct anatomical position.

The main difficulties in the cultivation of bioartificial organs lie in the fact that scientists cannot completely restore the architectonics of tissues. Cells are able to occupy the correct positions on a properly prepared decellularized donor ECM, but there are no technical solutions for occupying all the vacant cell sites or arranging all the necessary cells for a complete cellular kit of the organ. Thus, it is not possible to completely restore even the endothelium throughout the entire restored vascular bed. Since it is not possible to populate blood vessels more densely and evenly with endothelium, the start of blood flow in a new organ leads to contact of blood with collagen in exposed sections of the vascular bed. This contact activates the blood coagulation system, which leads to thrombosis, and the defective bloodstream of the bioartificial organ becomes clotted. Today, this complicates the implantation of tissue engineering products into a living organism and delays the prospects of introducing bioartificial organs into practical healthcare. On the one hand, as well as inside the vessels, living cells cannot be correctly and fully inserted into the organ parenchyma; therefore, the functionality of the first bioartificial organs is still low. On the other hand, there is no source or method for producing healthy cells in sufficient quantities to create new organs or parts of them in the laboratory. During cultivation, cells often lose their differentiation, and the number of cell duplications is limited.

An important issue is the cost of the technology. When growing pigment epithelium in Japan from induced pluripotent cells, Shinya Yamanaka noted the prohibitive cost – he estimated that several square centimeters of pigment epithelium obtained by reprogramming at approximately $ 200,000.

―We could start with human cells and the frame of a human organ or pig. However, obtaining the necessary reagents and growing a sufficient number of cells today will cost tens of thousands of dollars per one heart. It is too expensive to just get answers to our questions. Probably, we will slowly and gradually go forward, maybe growing only one heart at a time‖.

Liver

With prolonged washing of donor organs with such solutions, only the non-living part remains from the organ

– the EMC, or basically the connective tissue fiber backbone of the organ, sometimes called the skeleton of the organ. Since this skeleton repeats the shape of the organ and has a pale white color, such blanks in the West are often called _pale organ ghosts‘.

American specialists from the Massachusetts General Hospital under the direction of KorkutUygun successfully transplanted several rat livers grown in the laboratory from the own cells of the rats. For the first time, a viable liver transplant was built in the laboratory. A method was developed to populate a decellularized liver with living hepatocytes and vascular endothelial cells, which was evaluated by the researchers themselves as "the first steps in growing a working liver".

The experiment was carried out in several stages. First, endothelial cells were introduced into the liver using the preserved vascular frameworks, a little later about 50 million healthy liver cells of recipient rats were introduced into it, and a new organ was grown for another two weeks in the bioreactor. Thus, five new livers conditionally suitable for transplantation were created, bearing living cells that took their right positions in the structure of the connective tissue fiber framework. The organs were transplanted to the rodents and connected to their circulatory system. For eight hours, the livers organs showed vital functions.

Lungs

Laura Niklason, Professor of Biomedical Engineering and Anesthesiology at Yale University, a pioneer of vascular and lung tissue regeneration, head of her own laboratory,is known for creating the first bioartificial artery in 1999.In her laboratory, a working chamber for a tissue-engineered bioreactor for growing bio-artificial lungs was developed (6.37)

Thismatrix (a pale ghost of the lung (decellularized organ)) wasplacedinacultivationvessel,andamixtureoflungcellsobtainedfromnewbornratswas added. The most difficult thing was creating 3D cultivation conditions that simulate the conditions of the organ‘s natural development. As the cells took their places on the _skeleton‘, they began to divide and unite. After eight days of cultivation, they formed conditionally fully functional lung tissue.

The final proof of the proper formation of bioartificial lungs was obtained after a rat‘s left lung was removed and replaced with the grown organ. Special instruments recorded that the created lung inhaled and exhaled air, although not as completely as the neighboring, natural one. Testing the chemical composition of the blood entering and leaving the lung showed that the bioartificial lung gave oxygen and absorbed carbon dioxide with an efficiency of 95% of a healthy organ. However, the bioartificial lung functioned only for one hour*in vitro*.

Heart

In 2008, the Doris Anita Taylor (leading American scientist working in the field of regenerative medicine and tissue engineering, Head of research in the field of

regenerative medicine and head of the Center for Cell and Organ Biotechnology at the Texas Heart Institute in Houston) team published the results of a study convincingly showing that tissue engineering methods can produce a functioning organ – a beating heart from a suspension of heart cells and an ECM of this organ (35); these results have historical significance. After introducing the cell suspension into the matrix of the heart and maintaining the construct in the organ culture, it was possible to observe the organ self- assembly. Cells, when injected into the tubular cell-free structures of connective tissue fibers of decellularized vessels, independently occupy topographically correct positions in the organ‘s frame, and are able to establish interactions and restore heart functions again in the laboratory in a fairly short period of time.The experiments used the bioreactor of the American company Harvard apparatus created to grow a new beating bioartificial heart.

Precursor cells of cardiac tissue in this experiment were isolated from several hearts of newborn rats. Up to 106 cells were injected into one rat heart; the cell mixture included neonatal cardiomyocytes, fibrocytes, endothelial cells and smooth muscle cells. In a rat, a medium-sized heart weighs about 1.3 grams. For orientation in the numbers of transplanted cells, in the trachea prepared for humans (for Claudia Castillo), Macchiarini introduced about 60x106 cells, which is obviously too few for such a large organ as an adult trachea.

Thus, after four days of culturing cells on a matrix, Doris Taylor noticed muscle contractions. Eight days after the _skeleton‘ was colonized with cells, distinct heartbeat began. However, such heartbeat would only be enough to pump only 2% of blood that a healthy heart of an adult rat usually pumps.

In addition to this technological improvement, this work, undoubtedly, represents a breakthrough in the development of a more biocompatible and personified solution for the treatment of heart failure in clinical practice using its own natural framework as an initial matrix for autologous heart repair cells of the patient.

Numerouslaboratoriesareworkingoncreatingbioartificialorgansfromconnectivetissuefibersandlivingcells. Notably, in all the mentioned successful cultivation options, we are not talking about stem cells – the basis is differentiated cells. In all the cases, the necessary cell population for the successful creation of bioartificial organs is vascular endothelial cells(18).

To stimulate regeneration, scientists used transplantation in particular, yet with a different goal. Therefore, the Soviet professor Lev Vladimirovich Polezhaev proposed the concept of regeneration induction (31). If the individual is unable to independently restore the normal structure of a particular organ, an external effect is exerted on the regeneration process. Instead of the lost part of the organ, a regeneration inductor is introduced into the damage zone — these can be transplanted shredded tissue or organ fragments with or without living cells. In fact, specially prepared grafts-inductors are transferred to the trauma zone. A new part of the organ is formed instead of the lost one and is almost identical to it in structure. Thus, the integrity of a number of organs can be restored: the cranial vault in mammals and humans, muscles and retina in laboratory rodents.

Allostatic transplant regeneration was partially studied using transplantation of nerve cords, etc.

However, such newly formed, regenerated parts of organs are called regenerates, but not bioartificial organs
(21).

It should be noted that the organs of corpses are not quite accessible material; there are a number of legal problems that make their use difficult. Therefore, the role of Body Donation Programs and their further development is high.

3. The first transplanted hollow bioartificial human organs.

It must be emphasized that by 1960 in the USSR a clear idea was formulated by L.D. Liozner that a number of hollow (tubular) organs such as blood vessels, esophagus, trachea, and urinary tract have regenerative ability which manifests itself well if there is a support, a frame for tissue growth. At the same time, the dependence of the completeness and organotypic regeneration of these organs on the size of the removed organ site was emphasized, as well as the dual significance of a temporary prosthesis or graft – on the one hand, the prosthesis facilitates tissue growth, and on the other, it ensures the uninterrupted functioning of an organ, which enables this organ to exhibit regenerative ability (21). Liozner drew attention to the fact that the so-called replacement regeneration should be distinguished from regeneration by a prosthesis or frame (from polymer or non-organ materials) temporary or permanently established. By replacement regeneration, Liozner understood the gradual displacement of the transplant (allo- or even autologous) by the host tissues with the formation of the missing part of the organ (21).

Anthony Atala, professor of urology, Head of the American Institute of Regenerative Medicine at Wake Forest University, was the first in the world to create the bioartificial part of an internal organ (the upper segment of the bladder detrusor). Bladder tissue repair is often the only option for tissue loss due to cancer, trauma, or congenital malformations. The use of intestinal tissues for these purposes is still the gold standard in urological practice, but this leads to new problems: mucus production, stone formation and malignant tumors (1,2).

In 1999, Atala and his team of researchers transplanted to several patients a portion of the bladder grown using cell technology; it took about 6-7 weeks to create it in the laboratory. In one study, allogeneic cell-free bladder matrices were used to increase the size of this organ in dogs. The regenerated tissues had a normal organization, consisting of urothelium cells and smooth muscles. Biomatrices pre-seeded with cells prior to implantation showed better tissue regeneration compared to biomatrices implanted without cells, in which regeneration depended only on the ingrowth of the surrounding tissue. Biopolymers seeded with cells retained most of the implanted fragment, unlike implanted ones without cells, in which the graft was compressed and shrunk. Histomorphological tests showed a noticeable lack of muscle cells and a more aggressive inflammatory response in acellular matrices in another similar study.
To avoid these complications, Atala et al. investigated a scaffold-based bladder

repair technique using autologous bladder tissue for patients with poorly responsive bladders or high-pressure bladders. Based on the results of a bladder biopsy, a biodegradable scaffold in the form of a bladder was designed, namely a bladder template made from collagen and polyglycolic acid (GPA). The volume ofthe bladder was evaluated by morphometric analysis; the age of the patient and the size of the pelvic cavity were also taken into account. Fig. 1 shows the fabricated scaffold with seeded muscle and urothelial cells. The constructed bladder was implanted to repair the damaged organ.

The pilot study involved four girls aged 13 to 18 years. All of them suffered from a rare genetic disease - Mayer–Rokitansky–Küster–Hauser syndrome. This is a hereditary malformation in which the ovaries develop and function normally, the of secondary sexual characteristics are fully formed, but the uterus and vagina are not formed at all.

For the first time, four operations were performed with a vaginal transplant created from the patient‘s own genital organs. The results of 4–8 year observations of the operated women indicate a successful restoration of the lost function of the organs and are published in the *Lancet*.

The artificial vagina began to function normally, secreting mucus and lubrication, smooth muscles contracted painlessly.

Atala says that the three main obstacles are inability to grow the necessary cell populations *in vitro*, poor biomaterials and inadequate blood supply to the transplant.

It should be noted that initially, the scientific levels of technical solutions when creating the bioartificial organs of the above two groups of researchers were significantly different, not in favor of the team which included Macchiarini. However, in terms of press publications, probably due to the incompetence of the authors, the first place in creating new living organs in the laboratory was undeservedly awarded to the Macchiarini group which occupied themselves with bioartificial trachea. For instance, in 2008 The New Times wrote about this project: "The first successful transplant of an organ created from its own cells". In this and similar reports, the method for restoring the airways using the bioengineered trachea is positioned as a revolutionary breakthrough in the field of transplantology (23,24).

The main advantage and achievement of transplantation of bio-artificial organs, compared with the already known donor organs transplantation, is associated with the elimination of the need for pharmacological lifelong suppression of the immune system. Immunosuppressants are prescribed for life to all the recipients of foreign organs in order to preserve these organs in their bodies. Indeed, the suppressed immune system is not able to reject the donor organ, but the organ‘s performance becomes worse, and immunosuppressants themselves have side effects. All this causes gradual irreparable damage to the health of patients living with other people's organs – they have

impaired fat metabolism, an increased risk of developing cardiovascular diseases

and arterial hypertension, and the nervous system noticeably suffers. Over time, an increase in body weight occurs, steroid diabetes, osteoporosis,and renal failure develop, and later fatal complications often occur – malignant epithelial tumors and lymphomas. In general, transplantologists, while saving people, are not yet able to make them long-livers. Therefore, avoiding the need for constant lifelong administration of immunosuppressants is a long-standing dream of transplantologists and all of humanity, and fulfilling this dream will be a crucial step in the development of transplantology.

Thus, Macchiarini, together with colleagues, proposed two options for the design of the trachea with living cells to replace this organ. The first option is associated with a trachea from a deceased person, and only the cell-free part of the donor organ is used – all the cells are completely removed from the donor trachea using special solutions. The remainder is the tracheal matrix, that is, the ECM(mainly the fiber collagen backbone) used to colonize the patient‘s own cells and create an inferior bioartificial trachea which should, according to Macchiarini and his coauthors, regenerate independently to its normal structure inside the human body. The second option is not related to donor organs; instead of the trachea, an artificial tube of _nanomaterials‘ is transplanted which, according to the authors, should carry living cells on its surface and successfully replace the trachea (25).

In 2008, Macchiarini transplanted the first _bioconstruction‘ of the trachea, created in collaboration with scientists from different countries of Europe. Macchiarini ventured to use this design as a replacement for a person‘s main bronchus damaged by tuberculosis. How much this transplantation was justified isaquestionforexperts – thoracicsurgeons.

The main difference between the _new technology‘ and conventional transplantation was that the donor did not require a living or even a viable organ, but only its inanimate cell-free part. Usually, organs are removed from a potential donor after stating the death of the brain, while the heart continues to beat, and the organs of this person continue to live and function, supplied with blood that carries oxygen. This is a dubious ethical issue, but an organ taken by transplantologists after the cessation of blood supply is suitable for transplantation no longer than a few hours, then irreversible changes occur in most of the cells.

Since Macchiarini used only the devitalized (inanimate) part of the trachea, the solution of ethical problems and the timing before transplantation was significantly facilitated. When using the matrix, it is possible to take an organ from aperson several hours after biological death; therefore, there is no need to rush the organ to the recipient, and finding the donor-recipient pair suitable for immunological parameters is no longer a problem. On their surface, donor cells carry histocompatibility proteins by which the immune system of the recipient recognizes somethingforeign and seeks to destroy it, and in the laboratory, all the viable elements were removed from the donor trachea, and only its _skeleton‘ consisting of connective tissue biopolymers (ECM) was used.

4. Causes of failure with a 'bioartificial' trachea.

The problem of manufacturing implantable prostheses of hollow organs is at least a hundred years old. As early as 1911–1912, Alexis Carrel during an experimental operation on a dog replaced part of its artery with a rubber tube. The operation of stitching the ends of the arteries with the ends of this tube took Carrel almost four hours. He managed to provide a flow of arterial blood through the tube, and the prosthesis fully replaced a section of the artery. After 3 months, Carrel decided to check the condition of the prosthesis, and the second operation revealed that new living tissues were formed inside and outside the rubber tube. However, the conclusion was not entirely correct - the tissues on the outer surface of the tube correspond to the normal structures of vascular adventitia, and on the inner surface to the endothelial cells located on the normal connective layer. Carrel believed that a similar vascular prosthetics operation may be useful in medicine. A little later, this variant of regeneration was called tube regeneration, sometimes a less accurate definition was used – regeneration by frame or by prosthesis.

A large number of different variants of vascular prostheses were invented and are now successfully used to preserve the health of millions of people. The prostheses can be made of modern synthetic materials, for example, of porous polytetrafluoroethylene (Blood vessel prostheses ECOFLON, Russian-made), or bioprostheses, for example, of parts of the internal cattle thoracic artery, treated with an epoxy compound which provides better structural stability of the biological tissue and resistance to calcification and thrombosis (vascular bioprosthesis_KemAngioprosthesis', Russian-made). In general, the Russian Federation produces high-tech implantable prostheses, in these examples – vessels.

The commonest modern point of view on vascular prosthesis regeneration is the idea that neointima (pseudointima), a fibrous membrane that significantly differs structurally from the intact or normal endothelium with a basement membrane and underlying tissues, is formed inside the prosthesis

The leading Russian angiosurgeon, Academician Pokrovsky, does not share the widespread opinion that the endothelial lining is necessary for the full functioning of the vascular prosthesis. In practice, surgeons see that the prosthesis fully replacing the vessel copes with its functions, remaining _bare' inside, that is, without the endothelium. At best, a long-functioning prosthesis is lined with a layer of fibrin. Pokrovskyclarifies this observation is probably due to the older age of patients, when regeneration potential is reduced. At the same time, patients with this condition of prostheses live with them without complications for many years, sometimes decades.

Eveninelderlypatients,

theinternalareasoftheprosthesesborderingtheanastomosiscanhavepartialregeneratio noftheendothelium. It should be

emphasized that the trachea and large vessel are completely different organs in

completely different conditions – the properties of the media transported through these organs, and even their state of aggregation, are different. Blood is a conditionally sterile liquid suspension of cells, and air is a gas mixture, infected, carrying small foreign particles, etc.

In general, implants of different organs are characterized by common shortcomings long known to specialists. Firstly, the functions of a living organ can never be fully performed by the implant. Second, the duration of prostheses, sometimes referred to as _survival of prostheses‘, is usually inferior to their living regenerating prototypes. Implant prostheses are not capable of growth, self-healing or adaptation. There is always a biomechanical problem of joint tension between the prosthesis and the patient‘s living tissue. To some degree, an inter-surface reaction between living flesh and artificial material develops.

In Italy, for Macchiarini, when preparing the basis of the organ from the trachea using special simple technologies (some of them are used to extract DNA from tissues during genetic studies), all cells and even their DNA were removed, and the cell-free frame of the organ was populated by the recipient's own cells, taken from the patient in advance and multiplied in the cell laboratory. Unlike the press, we cannot consider this technology

_revolutionary‘ because scientists and research teams worldwide explored the possibility of transferring the matrix and living cells to the wound during several decades.

It should be emphasized that several laboratories in various European countries coordinating their activities were involved in preparing the donor trachea for transplantation. A 7 cm-long trachea section was taken from a 51- year-old donor. At the University of Padova,it was cleaned of donor cells using special solutions, and a connective tissue fiber skeleton of the trachea was obtained for further modification - colonization by Castillo‘s own cells.

In the UK, from the bone marrow previously taken from Castillo, plastic-adhesive (adherent to the bottom of the plastic culture bottles) stromal cells were isolated which are now called multipotent mesenchymal stromal bone marrow cells. Martin Birchall, professor from University College London (UK), funded the reproduction of these cells. This is a serious structure with good funding, able to quickly and efficiently develop and implement breakthrough improvements in medical technology. Burchall is also known for performing the world's first combined larynx and trachea transplantation at the University of California in October 2010 (5,7).

According to Burchall, in an experiment on pigs he developed a strategy for using organ-deprived cells in the form of connective tissue fiber cores for their transplantation in combination with own cells, including stem cells (differentiated or undifferentiated) (5). The culmination of such developments was the world's first transplant of bioartificial organs – trachea, created on the basis of stem cells, to an adult (Lancet, 2008) and to a child (not published, March 2010).

An important feature of the transformation of any matrix from connective tissue fibers in a living organism should be noted, which dilettantes are usually not familiar with: all biopolymers dissolve and are replaced by regenerative connective tissue, therefore, it is completely insufficient to put a framework even to recreate, preserve or change the shape of organs.

Professor Anthony Hollander from the University of Bristol carried out the preparation of cell cultures for the construction of the trachea. Hollander himself is pleased to note that he was the only stem cell specialist in the research program that ended in Barcelona with transplantation of Claudia Castillo‘s bio-trachea (17). With the help of the long-known and well-known differentiation inducers (or differentiating signals), Hollander transformed Castillo's culture of multipotent mesenchymal stromal bone marrow cells into a culture of chondroblasts (cartilage cells). Notably, Hollander specialized in cartilage cells, and his professional interest prior to working with the trachea lay in the restoration of articular cartilage.

Cartilage cell culture, obtained by Hollander, was transferred to the donor connective tissue fiber frame of the trachea, placed in a two-chamber rotational bioreactor, specially created at the Milan Polytechnic University of Italy by Sarah Mantero for growing tubular hollow organs. The bioreactor itself was positioned as a device that allows creating two chambers for growing different cell populations in a hollow tubular organ. However, to maintain the growth of epithelial cells and cartilage, completely different and specific culture media and cultivation conditions are needed that are used only separately for each differential cell.

The cultivation of human progenitor cells of the epithelium of the bronchi and trachea is carried out at the interface between the aqueous and air environments (air-liquid interface, ALI). It is quite obvious that the porous tracheal frame cannot be a reliable distinguishing barrier between different solutions, and the division of media for the respiratory epithelium there is quite different. Two days (during which the cells of these two populations were in the same bioreactor) is completely insufficient for a significant increase in their cell mass. It is possible that during this period some cells managed to adhere to the connective tissue skeleton of the trachea, but no scientific information or photo documents showing what happened to the cartilage and epithelium cells under such conditions were presented. It is not clear whether such cells can even divide within this bioreactor.

Strictly speaking, Macchiarini himself did not participate in the most important process of creating a new trachea, since he did not possess the necessary special knowledge and skills. Macchiarini says: —We took the donor trachea, cleaned it of the cells, took undifferentiated stem cells from the patient‘s bone marrow, placed them in a nutrient medium and differentiated using the growth factors in the cells that we needed (for the external part of the trachea). For the inner part of the trachea, cells were taken from the nasopharynx and from the trachea itself and also
propagated. We prepared the matrix in Barcelona, cells were grown by specialists in Bristol (chondrocytes and cells of the respiratory epithelium), and the

bioreactor was in Milan. After everyone did their job, the transplant took place in Spain. Everything went brilliantly, but all this is too complicated to be able to help everyone‖.

In fact, preparation of such cellular material (originally obtained from the human bone marrow) is not complicated, and in case of such _assembly, the term _biotrachea' is not applicable. The laboratory where the author of the present research work at is fluent in such technologies, and their cost is much lower than the amount announced in the press (a million USD). Moreover, numerous questions to the technology arise that cast doubt on even the theoretical possibility of achieving _brilliant' results.

Firstly, it remains unclear why preclinical trials of such bioengineered tracheal structures were not conducted before transplantation to a living person. Tracheal transplantation is an extremely risky operation, and errors lead to asphyxiation, infection of the mediastinum with a risk of damage to vital organs and blood vessels, and to life- threatening breathing difficulties. Macchiarini claimed that he conducted preclinical trials of the bioartificial trachea in mice and pigs providing no evidence of these. The structural dynamics of recovery processes with this technology and the true role of the cells used were not studied and are incomprehensible.

Second, the choice of the trachea to replace the main bronchus causes some bewilderment, since the structure of these parts of the air ducts is noticeably different. In the trachea, hyaline cartilage is presented in the form of open semi-rings, and a membrane of connective tissue with smooth muscle fibers that form the so-called tracheal muscle represents the back of the trachea. In the main bronchus, hyaline cartilage is in the form of rings, and muscle fibers form a circular plate. The biomechanics of these anatomical formations are different even if only the matrices of these parts of the airways are used. In sum, no justification was given for replacing the bronchus with the trachea.

Third, although mentioned, stem cells as they are were not actually used for the trachea in Castillo's case. In laboratory conditions, plastic-adhesive fibroblast-like multipotent stromal bone marrow progenitor cells were isolated and propagated from the bone marrow, the culture of which was gradually transformed into cartilage-like cells under the influence of differentiation inducers. 60 million cells were introduced into the tracheal frame, and it is not specified whether it is fewer or more than the number of hyaline cartilage cells normally present in the trachea. For reference: there is evidence that an average human cell 10 μm long weighs about 1nanogram, so for the trachea, the indicated number of grown cells is too small.

Moreover, transplantation of chondrocytes alone to restore hyaline cartilage is perplexing. A distinctive feature of cartilage is the absence of own vessels, and it is well known that hyaline cartilage can exist only surrounded by a perichondrium rich in blood vessels, or in a joint constantly washed by synovial fluid that feeds it. Moreover, for the normal state of articular cartilage, the limb must be constantly moved. The perichondrium is a connective tissue membrane covering the cartilage and consisting of two layers – the outer (fibrous) and the

inner (chondrogenic, cambial), penetrated by a large number of vessels. The poorly differentiated cambial (sprout) cells are mainly localized around the vessels, and upon specialization, they turn into chondroblasts and chondrocytes. That is, without the perichondrium, cartilage regeneration will not occur in the trachea. If the perichondrium is kept and the hyaline cartilage is removed, for example, in the costal cartilage, this part of the rib will independently and completely recover after some time. Contrariwise, the cartilage will slowly dissolve. Therefore, plastic surgeons always use hyaline cartilage along with the perichondrium for transplants. Chondrocyte transplantation without restoration of the perichondrium and without ensuring blood supply in it with the hope of spontaneous restoration of the cartilage rings of the bronchus in the recipient's body is autopia. This was indirectly repeatedly confirmed in numerous studies, including the group of Japanese professor Kojima in attempts to create cartilage using chondrocytes and fibroblasts. Without reproducing the conditions of a suitable microenvironment and nutrition, no cartilage can exist by itself. Moreover, the hyaline cartilage of the trachea is represented by cartilage and perichondrium, and science cannot yet grow a new full-fledged perichondrium.

Fourth, two populations of cells - the epithelium of the respiratory tract (epithelial cells obtained from the lining of the nasal cavity) and cartilage cells - are insufficient to create a functioning trachea. Smooth muscle cells are essential to ensure the mobility of the trachea and the stability of its shape. Without smooth muscles, neither the trachea nor the bronchi can maintain their shape and fulfill their functions. In the membranous part of the trachea, there are smooth muscle fibers also passing into circular ligaments. Due to this, the lumen of the trachea narrows when people exhale and expands when they inhale. This is important for coughing – scientists determined the physical properties of the back wall of the trachea using mathematical modeling and discovered that the point of coughing is to clear the lungs and airways. All foreign bodies and waste products come out with mucus, and during coughing mucus is exuded more efficiently if its layer on the walls of the trachea is thicker and the airflow is faster.Preferably, the air velocity in the trachea during a coughing act should change, and this is possible due to the elastic back wall. When coughing, its muscles contract, and the lumen of the trachea narrows by twenty percent. As a result, both the airflow rate and the thickness of the mucus on the walls increase. In the congenital absence of smooth muscle tissue, a pathological expansion of the airways occurs, and problems arise with the respiratory

system. In this case, the inferior malleable walls of the trachea and bronchi are stretched under the pressure of the inhaled air, and the lumen of the tracheobronchial tree significantly increases compared to the norm, causing the development of diverticula and expansion of the trachea up to tracheobronchomegaly, which leads to lung diseases. The cells of numerous glands of the trachea are of great importance, and the conditions of growing them outside the body are known and significantly differ from the cultivation of the epithelium of the respiratory tract. Without organotypic fibroblasts, the trachea will never restore a normal fiber backbone, etc.
In almost two decades of trying to create a bioartificial organ, Professor Anthony Atala found that it is optimal to use the matrix together with

endothelial cells and vascular growth factor (VEGF), since the latter provide the adaptation and germination of new cells in the body. Macchiarini used neither endothelial cells nor such growth factor to create a _bioartificial' trachea.

Fifth, reparative regeneration of the mucosa and submucosa of the trachea with its many mucous-protein glands is poorly studied by histologists – possible extent and conditions of restoration of these structures are not clear. Researchers believe that regeneration of the respiratory epithelium is possible only at a small distance from the edge of the wound of the mucosa, and with significant full-layer circular defects, the restoration of the inner layer will not occur. Normally, a special layer of mucus protects the ciliated epithelium of the respiratory tract in humans. The cilia of the ciliated epithelium normally function in a double layer of mucus up to 6 microns thick, secreted by the goblet cells of the trachea. The lower layer is the sol in which biologically active substances, enzymes and immunoglobulins are located, and the concentration of immunoglobulins is almost 10 times higher than in the blood. The upper layer of mucus is a viscous-elastic gel touched only by the top of the cilia and performing mainly a mechanical function. In the human body, 12,000 liters of air pass through the trachea into the lungs during the day. In order to prevent drying of the mucosa and, especially, of the underlying tissues of the walls of the trachea, several protective mechanisms appeared during evolution that were ignored when this organ was recreated artificially. The capabilities of cell technology to restore the new inner layer of the trachea remain unclear.

Sixth, the issue of blood supply to the _grown' trachea was not thought or resolved. All cases of successful transplantation of a donor trachea were associated with a more or less adequate restoration of blood flow in this organ, yet tn the Macchiarini technology, no vessels were provided for the bio-artificial trachea at all.

Macchiarini claimed that the trachea is a hollow organ with a simple structure, implying it can somehow recover by itself; however, this is highly unlikely – trachea as an organ is not capable of marked reparative regeneration. More than two centuries ago, the Swiss natural scientist Charles Bonnet rightfully stated that regeneration is a form of adaptation, and organs that are most likely to suffer loss or damage under natural conditions regenerate best. It is clear that the trachea is a vital internal organ, and its significant damage in nature is incompatible with life. It is doubtful that in the course of evolution there was a selection of animals according to the ability to regenerate this organ (however, a pronounced regenerative ability may manifest in the epithelium of the respiratory tract).

New arteries of a large diameter formed by reparative regeneration to restore sufficient blood flow in the trachea are also unlikely – as a rule, the human body is not capable of spontaneous reparative regeneration (under ordinary conditions) of large vessels, and this issue requires further studying. The secondary development, growth and functioning of new large vessels is possible in humans during pregnancy, tumor processes and in some varieties of pathological or atypical regeneration. It must be borne in mind that the trachea is an organ of

complex structure, designed for hard and constant work;therefore, it urgently needs an intensive blood flow. The need for good blood circulation is associated with a large amount of air passing through the trachea while breathing. To protect the mucous membrane of the trachea from drying out and to sufficiently moisten the inhaled air, serous-mucous glands and goblet cells producing mucus actively function. The blood flow is also necessary to ensure active movements of the tracheal muscle and smooth muscle bundles in it, as well as of the cilia in its mucous membrane.

The trachea has another extremely important function – conditioning, that is, processing the inhaled air before it enters the lungs – it must be warmed and moistened, and foreign particles must be removed from it. The human respiratory tract is constantly _washed‘ with the normal secretion of the tracheobronchial tree. This secretion is viscous semi-liquid mucus with a bactericidal effect, containing antimicrobial protective molecules, namely lysozyme, immunoglobulins, and cellular elements (alveolar macrophages and lymphocytes). Tracheobronchial secretion helps remove inhaled foreign particles, cellular detritus and certain metabolic products. Normally, the volume of tracheobronchial secretion does not exceed 100 ml per day and is swallowed by a person when excreted. There is even a special term –mucociliary clearance. A quick push of the cilia upward with a beating frequency of about 1000–1300 per minute ensures sputum movement at a speed of 0.3–1 cm/min in the bronchi and 2-3 cm/minin the trachea. Congenital and acquired pathology of the ciliary epithelium leads to the development of obstructive pulmonary diseases currently understood as progressive and characterized by an inflammatory component, impaired patency at the level of the distal bronchi and unfavorable structural changes in the lung tissue and blood vessels. In addition, bronchial obstruction develops with fixed cilia syndrome. Even ignoringMacchiarini and assuming the trachea is assembled perfectly, without adequate restoration of the vessels, their architectonics and sufficient blood

flow through the organ, all efforts will be in vain. Already 6 hours after the blood supply in the trachea stops, irreversible changes begin andproceed even quicker in case of thermal ischemia.

Seventh, Macchiarini definitely stretched the truth speaking about the beneficial effects of the operation:

—The results exceeded all expectations – there was no rejection reaction, the woman fully recovered. Moreover, blood vessels began to form rapidly on the integrated part of the trachea, which we did not dare to hope for ‖. However, it should be noted that _fully recovered‘ is a strong exaggeration in Castillo‘s case. The persistent pathological growth of granulation tissue at the site of the proximal anastomosis was a serious long-term problem for Castillo's life and health – she was regularly hospitalized every 3 months for bronchoscopy. Sometimes, the lumen of the bronchus was expanded by installation of stents at different intervals as the narrowing of the trachea developed. It is not clear whether Castillo currently has a bronchial stent or whether she is still taking antibiotics and other medicines, and what the condition of her lungs is.

The whole absurdity of the situation lies in the fact that Macchiarini is not a specialist in the field of cellular technologies and cell biology, and knowledge of these sciences is the basis for successful creation of bioartificial organs. All of Macchiarini's activities in the project were mainly reduced to coordinating actions and implanting a certain semblance of a tube – a treated donor trachea – instead of a bronchus, which is not a significant difficulty for an experienced thoracic surgeon. It should be emphasized that Macchiarini did not have a successful experience of tracheal allograft in humans.

However, Castillo survived the operation, her lung straightened, and the woman began to breathe freely through this _bronchus‘, which caused a surge of optimism among the participants in her treatment.

Further events were of great interest. According to Macchiarini, the technology that was implemented in Castillo‘s case is too complicated and costly (the sum of about 1 mil USD was announced). Therefore, Macchiarini‘s team decided to simplify the process to make such technologies available to everyone, which, according to Macchiarini, meant an amount of 60 to 130 thousand euros for each new operation. The spread in costs depends on the diagnosis and the associated standard treatment. Simplification implied excluding the stage of laboratory preparation and growth of cellular biomass to create a bioartificial trachea, as well as eliminating the stage of preliminary _growing‘ this trachea in a special bioreactor before transplantation into the human body.

Reasonable minds wondered whether the scheme is too simple, and Macchiarini's answer was puzzling: —It is just like with women –would you want your woman to be simpler and more accommodating, or vice versa? Simple technologies will work‖. The absurdity of such answer gives an impression that Macchiarini has no idea on how organs regenerate in nature at all. Neither epimorphosis nor morpholaxis are possible in humans. Regeneration induction, regenerative hypertrophy, allostatic transplant regeneration – it is not clear what new mechanisms unknown to regeneration specialists can work to recreate the trachea in this way.

Macchiarini announced the creation of a new concept of bionic treatment or bionic therapy. The principle of this _new concept‘ is to replace damaged organs or tissues with new ones. Macchiarini coined another new term,

_bionic regeneration‘, to explain how treatment is effectuated –__a damaged human organ itself serves as a reactor for growing the affected areas. After all, what could be better than using your own body forces? The idea is to activate stem cells in the patient‘s body, so that the body itself recognizes where it is necessary to repair the damage, and does it‖. Strictly speaking, this concept shows all the signs of a pseudoscientific research, and new intricate, previously non-existent terms are a proof in this case. Pseudoscientific studies are usually motivated by the achievement of an immediate, practically useful result; they demagogically appeal to scientific methods while only imitating them – these are all sorts of new scientific hypotheses that contradict firmly established scientific facts. A pseudoscientific theory is created by a person or a group of people who are not specialists in the relevant field. It should be specifically emphasized that

regeneration of organs or their assembly at the molecular and cellular level is the subject of the following scientific specialty in medicine – _Histology, Cytology, and Cell Biology‘, and not surgery. Other signs of pseudoscientific research in Macchiarini‘swork are as follows: claiming the approach is revolutionary inscience and technology and ignoring or distorting facts that are known to the author but contradict his theoretical constructs. The author actively uses the theory to conduct personal business; further on, proof will be given of all these signs of pseudoscientific research.

What is bionic regeneration according to Paolo Macchiarini?In general, regeneration is a combination of biological processes aimed at restoring damaged or lost structures in a living organism.

The adjective _bionic‘ means that regeneration is related to bionics; however, unlike regeneration bionics isnot a process but a science that is a borderline between biology and technology, solving engineering problems based on modeling the structure and vital functions of organisms. Bionics can also be defined as the doctrine of the methods for creating technical systems with characteristics close to those of living organisms. Finally, bionics can also be defined as a section of cybernetics that studies the structure and vital activity of organisms to solve engineering problems. Therefore, regeneration cannot be bionic; such assumption is absurd and contrary to elementary logic.

Directly in the operating room, Macchiarini introduced a cell suspension into the walls of the prepared tracheal matrix. He later explained that stem cells were seeded from the child‘s own bone marrow onto the donor trachea along with growth factors: —We forced the cells to differentiate and transform, in a natural way, into cells of

all layers that make up the airways‖. There are many inaccuracies in this statement – firstly, these were not stem cells that were transplanted but a mixture of different bone marrow cells (usually, they are referred to as mononuclear bone marrow cells) among which there were different stem cell populations but only in very small quantities. Second, only 2 growth factors were used – Granulocyte-macrophage colony stimulating factor (G-CSF) and erythropoietin (EPO). These drugs are approved for clinical use and affect hematopoiesis –they stimulate the formation of certain bone marrow and blood cells. These are most often used in oncology but these cytokines are generally not necessary as differentiating signals in the case of the trachea, that is, they do not have the ability to differentiate bone marrow cells into the cells of which the trachea actually consists. Moreover, these cytokines cannot directly support the growth and differentiation of the necessary cells in the new trachea. The author of the present study believes the choice of these pharmacological preparations was due to rather simple considerations imitating scientific nature:

1) accessibility,

2) their effect on stem cells, namely, hematopoietic cells (G-CSF upon
3) administration, causes mobilization of bone marrow stem cells),

4) the available permissions for the clinical use of these cytokines,

5) an attempt to justify the possibility of converting bone marrow mononuclear cells into the cells that neither practically nor theoretically can be converted from 99.9% of the bone marrow cells. The effects of G-CSF and EPO on reparative tracheal regeneration have never been investigated, andthe reparative regeneration itself of the trachea at the tissue level by morphologists has not been seriously studied.

Macchiarini stated: ―In our first observations, we sowed epithelial cells obtained from the lining of the nasal cavity on the inner surface of the graft. However, from experiments with animals, it became clear that this was not necessary; bone marrow cells colonizing the carrier wall migrate and differentiate independently. To obtain the epithelial lining of the tissue-engineering structure, it is enough to sow the entire artifact matrix with bone marrow cells‖. This statement is completely false – it has never been documented either in animals in an experiment or in humans in clinical studies that bone marrow mononuclear cells can independently form the mucous membrane again from scratch, moreover, with ciliated epithelium and submucosal layer. This is impossible in the trachea and when performing this ―technology‖. Macchiarini constantly claims the presence of epithelial progenitor cells in the bone marrow, which is a very controversial statement despite a number of well-known publications. For instance, in 2006, scientists from the Yale University School of Medicine (USA) showed that part of the bone marrow stem cells after transplantation differentiates into functioning cells of the intestinal epithelium. In 2007, Employees of the University of Minnesota (USA) John Wagner and YakubTolar in collaboration with colleagues from universities in other states and in Great Britain and Japan, managed to find a rare population of cells in the bone marrow that had the ability to

_repair‘ the skin (epithelium) in laboratory models. This allowed justifying the need and transplanting donor bone marrow in children with severe hereditary pathology, known as recessive dystrophic epidermolysis bullosa (RDEB). Thus, scientists were able to achieve a significant increase in the concentration of normal collagen-7 in the skin and gastrointestinal tract in patients, save the lives of such children and prevent the destruction of their skin and upper gastrointestinal tract.

Tracheal prosthesis from "nanocomposite,Macchiarini-Seifaliandesign".

Thus, an Icelandic student, a 36-year-old man from Eritrea, Andemariam Teklesenbet Beyene, was sent to Stockholm for the cultivation of a new trachea. The well-known Icelandic surgeon, Professor-oncologist *Tomas Gudbjartsson* from the Landspitali Hospital of the University of Iceland no longer had the options to save this patient. Beyenehad an extensive tumor sprouting into the walls of the trachea and spreading to the junction of the bronchi. Thus, surgical removal of the tumor was impossible, since it was necessary to remove the trachea with part of the bronchi, after which it was impossible to ensure and maintain breathing. Other types of anticancer treatments (chemotherapy and radiation therapy) could no longer stop the

growth of the tumor. When the tumor grew to the size of a golf ball, it began to restrict breathing, and the patient could soon die from suffocation.

Macchiarini decided to transplant a new tracheal design: ―The main distinguishing feature of the new method is constructing cell scaffolds on which stem cells are deposited, creating an exact copy of the original organ. With transplants of this type, no immune rejection of foreign tissue occurs, since the transplanted organ consists of the tissues of the patient. There is no need to take dangerous drugs that suppress donor organ rejection‖.

However, such statements are highly doubtful –anexact copy of an organ is not possible since the structure of this implant or prosthesis is completely different from that of a live trachea; is the shape of the prosthesis being identical to the trachea is also very dubious. The transplanted organ did not consist of any tissues, since only cells were planted on the synthetic frame. Moreover, these were poorly differentiated and mainly hematopoietic – not the main type among the cell populations of the trachea. In 2 days, no new tissues will be able to form during cultivation even from the necessary cells of the primary culture. Since the prosthesis is made of bioinert materials (even if it carries a certain amount of living cells of the patient), mentioning the immune rejection and immunosuppressants is irrelevant.

On July 8, 2011, the BBC Russian released the following news: "The world's first transplant of an artificial organ was performed". However, transplanting artificial organs for several decades has been a very common

medical procedures (dental implants, artificial joints, blood vessels, heart valves, etc.). Even more complex in structure, living bioartificial organs had been transplanted before Macchiarini, andby 1999 convincing data was obtained on the success and effectiveness of such operations. Moreover, the bioartificial parts of the organs were transplanted repeatedly to patients with different pathologies.

What was actually done to save Beyene? 3D-imaging (digital technologies of forming and processing a three- dimensional image) was used when working with medical imaging data of the neck and chest organs of this patient, and later transferred to England from Iceland. Using rapid prototyping, a glass model that imitates Beyene‘s tracheal lumen was created, but it was not explained how the healthy form was reproduced, given the destruction and deformation of the trachea by the tumor in the patient. The model was covered in a special _nanocomposite polymer‘ invented in the laboratory of Professor Alexander Seifalian (7). Polymer production technology and the method for trachea prosthesis construction are Seifalian‘s intellectual property. That is, the prosthesis was formed in laboratory conditions, taking into account all the individual anatomical dimensions of the trachea required for Beyene.

For the tracheal prosthesis, the _correct‘ properties of the material are very important. Possibly, the

_nanomaterial‘ itself was successfully selected, and the prosthesis walls turned

out to be elastic with a spongy structure, yet such prosthesis cannot be a full-fledged part of a bioartificial organ for well-known reasons – the popular principle absorbable scaffolds was not used.

Further, the implant was sent by plane to Sweden, where Macchiarini immersed it in a bioreactor, having previously applied the patient‘s bone marrow cells to the product. The prosthesis was inside the bioreactor for about 2 days – not enough for the primary bone marrow cell culture to actually form something. It is completely unclear how the cells interacted with the new material. A competent morphological study of the structural dynamics of cells on the tracheal prosthesis was not performed, and there was no sufficient evidence prosthesis by the scientific community of the presence of living cells on this.

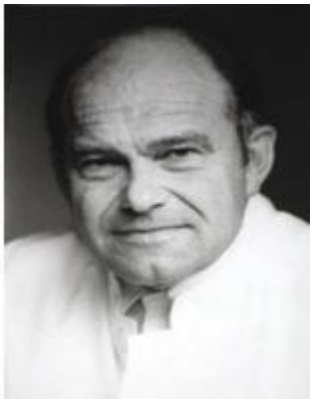
The resulting product is often called the _first synthetic trachea‘, which is inaccurate. For instance, in 1957– 58in the Karolinskahospital of Stockholm, S. Ekestrom and E. Carlens used a Teflon prosthesis tube to replace a tracheal defect in humans (Teflon prosthesis in tracheal defects in man. ActaChirScand Suppl. 1959; 245: 71-5); the patient lived with this synthetic trachea for at least 1.5 years. The Teflon mesh prosthesis also carried the patient‘s own cells – for several weeks, the Teflon mesh was attached to the front sheet of the *vagina m. recti abdominis*of the patient, and young connective tissue with vessels grew into it. In fact, Teflon is a polymer of tetrafluoroethylene, a commercial product obtained by chemical synthesis (synthetic material)first used for prosthetics of a trachea almost 50 years ago.

Paolo Macchiarini stated: ―Thanks to nanotechnology, using stem cells we can _grow‘ an artificial trachea or other organs in two days or maximum in a week and no longer need to wait for the donor. Further, in just two days, millions of pores in the artificial trachea were filled with the cells which quickly grew and formed tissue that was genetically no different from the patient‘s natural tissue‖. Such statement is not only populist but also deceitful – cells develop in close contact with the ECM that has the vital influence on their division, differentiation, maintenance of a specialized structure, movement, etc. This is a fact established and accepted by the scientific community. Growth factors (cytokines) are no less important for proper regeneration and maintenance of the organotypic structure, and their synthesis is highly influenced by the intercellular environment.

The destruction of the architecture of any organ, including obtaining primary cell cultures, is accompanied by a violation of intercellular interactions and the interaction of cells with the matrix, and most importantly, causes massive changes in the transcriptional program of cells which normally ensures the maintenance of specific or organ features of the structure and functions of cells. Changing the properties of the ECM changes the behavior of cells and even the expression of their genes, including those associated with the secretion of the necessary levels and growth factors that are correct for a specific part of the body. Not all the subtle mechanisms of maintaining the normal structure of organs and tissues are still clear, yet it is clear that normal tracheal tissue cannot be grown on or inside a tube from some unnatural and non-absorbable _nanomaterial‘ inside a living organism. This is excluded, both theoretically and

practically (26, 36).

5. Competing surgical technologies for the restoration of the human trachea.
It is likely that the first successful trachealallotransplantation in man was carried out in 1979 at the University Hospital ofCologne (Germany) by a group of German surgeons.



Fritz Wustrow, *Professor of Surgery, Head of the University Hospital of Cologne*

Kurt Guenther Rose,*Professor of Surgery of the University Hospital of Cologne*

German surgeons transplanted a live trachea in two stages. First, a pocket was made in the sternocleidomastoid muscle of the recipient‘s neck, into which the trachea was inserted so that the vessels of the muscle grew into the walls of the trachea and began to supply it with blood. A similar technique today is often called prefabrication. Then, after three weeks, when the new vessels had already grown into the organ and the blood circulation of the foreign trachea in a new unusual place was restored, they proceeded to the second stage. During the second operation, the tracheal transplant was isolated along with a part of the muscle, and a vascular pedicle was formed from the own vessels of the muscle, through which blood flew into the transplant. The whole complex of foreign and own tissues was transferred to its proper place (orthotopically) instead of the patient‘s own trachea– narrowed and unsuitable for breathing, and excised by

surgeons. Due to the formed vascular pedicle, it was possible to ensure the flow of blood to the trachea, which was necessary for its successful implantation. The operation was successful, breathing with the help of a new trachea was restored, and the article of 1979 reported that at the time of publication (17 months after the operation) the patient felt fine.

Most interestingly, Russian surgery has a certain priority in tracheal transplantation. In 1990, Soviet thoracic surgeon Yu. N. Levashov successfully transplanted the entire thoracic section of a donor trachea devoid of its own vessels to a 24-year-old female. A specific feature of transplantation according to Levashov was the simultaneous operation, that is, part of the donor trachea was stitched into the correct (orthotopic) position immediately. To prevent ischemia of the transplant, it was immediately surrounded by thegreateromentum of the patient (recipient) displaced from her abdominal cavity during the operation. Notably, surgeons generally often use the greater omentum during reconstructive operations – this easily moveable fold of the peritoneum can be stitched to any organ to preserve it. The omentum is rich in vessels, which, receiving signals of ischemia, quickly grow into the organ, while the newly formed vessels growing from the omentum quickly create an arterial flow and outflow of venous blood, saving the organ from ischemia and death. Surgeons know the antimicrobial, immunogenic, plastic and other useful properties of the omentum, which turned out to be very useful for tracheal transplantation. Levashov emphasized that he always used plastics with theomentum during thoracic operations with an increased risk, providing _tightness‘ of the walls of the airways. Thanks to this surgical technique, in right-sided pulmonectomy (removal of the entire lung on the right) the frequency of the bronchus stump insolvency was reduced five times. In Germany, thoracic surgeons also used the large omentum for lung transplantation at that time.

In 1997, at a meeting of the Pirogov National Medical and Surgical Center in Moscow, Yu. N. Levashov and co-authors from the State Scientific Center for Pulmonology of the Ministry of Health of the Russian Federation presented a report on the importance of the greater omentum for reconstructive operations on the trachea and in the resection of most of it. Thanks to perfect mastery of surgical technique and unique properties of the omentum, it was possible to remove most of the pathologically altered trachea in patients and restore breathing without resorting to transplantation and the use of donor organs. Levashov presented the results of the treatment of patients who

underwent a circular resection of a trachea (most of the organ was removed) with simultaneous connecting of the greater omentum on the pedicle to the anastomosis (to the junction of the remains of the patient‘ss own trachea). For instance, patient B., 43 years old, underwent the removal of the thoracic section of the trachea - 12 half rings (the trachea contains from 16 to 20 cartilaginous hyaline half rings in total) affected by adenocystic cancer. In the postoperative period, a 2-cm divergence of the tracheal joint with the ends of the respiratory throat developed, but due to the omentum, the airways were completely sealed. The cicatricial process in the area of the stitched upper and lower ends of the trachea narrowed the respiratory lumen (granulation stenosis) but was timely

eliminated after endoscopic procedures - the newly formed connective tissue in the lumen of the trachea was removed with a laser, and the lumen was additionally expanded with two endoprostheses from the inside. The patient breathed normally and was discharged home for furtheroutpatient monitoring. The second example cited by Levashov, patient L., 16 years old, underwent excision of 8 narrowed and severely deformedrings of the thoracic section of the trachea; the irreversible destruction of the trachea was caused by a prolonged use of the respiratory tube for artificial lung ventilation when the patient was in a coma. Despite the pronounced pathological densification of the tracheal walls due to scarring, and the significant mechanical tension of the tissues that occurred after the operation in the anastomosis zone, the postoperative period proceeded smoothly, largely due to the omentum. The young man was discharged on the 17th day after the operation, and long-term prognoses were favorable.

In turn, Harvard experts (Malcolm M. DeCamp, Professor of Thoracic Surgery at the Center for Chest Diseases at Harvard Medical School) showed that if a longer section of the aorta is taken, acceptable airways do not form; in other words, it was once again confirmed that during the restoration of the respiratory tract, the extent of the defect becomes very important (38).

The idea of using the greateromentum to restore the airways is interestingly developed abroad. In March 2003, the Austrian transplant professor W. Klepetko(Vienna Medical University; Department of Thoracic Surgery)published the long-term results of a complex two-stage operation to restore the trachea combined with lung transplantation. A 57-year-old suffocating patient with advanced chronic obstructive pulmonary disease plus a simultaneous narrowing of the trachea underwent standard bilateral sequential lung transplant. To avoid excessive complication and prolongation of the operation, it was decided to use the trachea from the same lung donor. At the first stage, the trachea was temporarily placed in the abdominal cavity, wrapped in the greater omentum of the patient, and the ends of this trachea tube were stitched to the abdominal wall, creating stomas. The patient received immunosuppression consisting of cyclosporin A, mycophenolatemofetil and cortisone.

Sixty days after the unusual transplantation into the abdominal wall, the study of the trachea showed that the allograft had a normal appearance both macroscopically (the elasticity and rigidity of the hyaline half rings of the trachea was preserved) and microscopically (the normal structure of the walls). Six months after the lung transplant, the patient‘s trachea and cricoid arch were removed, and the airways and normal breathing were restored thanks to this foreign trachea previously prepared in the patient‘s body. It was concluded that the foreign trachea, wrapped in theomentum in the abdominal position, retains functional and structural integrity and allows for delayed successful reconstruction of the airways by applying a direct end-to-end anastomosis between the remnants of own trachea and the prefabricated part of the donor.

The biopsy of the tracheal wall revealed that the trachea looks healthy, the mechanical properties of the organ walls are completely preserved, and the cartilage is covered with epithelium of the respiratory tract. Moreover, the

tracheal wall was perfectly vascularized (had many active vessels with active blood flow). At the time of publication, the patient had been living for 31 months after transplantation. Levashov‘s data were confirmed that the donor trachea wrapped in the recipient‘s omentum retains its functional and structural integrity and can be successfully used for two-stage allotransplantation.

Trachea transplantation does not always require lifelong administration of immunosuppressants. Almost simultaneously with Macchiarini, Belgian surgeons developed a new method for transplanting part of the donor trachea, which allows the trachea to be restored and lifelong medications to prevent transplant rejection to be lifted. Patients who are not suffering from malignant tumors can recover their breathing with high efficiency and quite safely.

Russia has a certain priority in basic researchin the field of tracheal anatomy and morphological features of its blood supply as well. At the Department of Topographic Anatomy of Sechenov University, Sergey Dydykinpresented his doctoral dissertation in 2001, having carried out extremely important and original studies on the topic ―Topographic and anatomical justification of allotransplantation of the trachea on the vascular pedicle‖. One of the practically important conclusions was made about the presence of a ‗lateral vascular pedicle‘ in the trachea, which can be used by surgeons to effectively restore blood circulation in the donor trachea immediately after transplantation.

Sergey Sergeevich Dydykin, MD, Professor of Sechenov University

Dydykin developed in detail the surgical techniques for preparing the donor trachea for transplantation, with the possibility of the fastest restoration of blood circulation in the transplanted organ. He proposed the technique of

‗quick‘ tracheal removal – the trachea is removed as part of the donor complex along with the muscles, vessels and fiber of the mediastinum, which reduces the period of thermal ischemia and the number of dead cells. Dydykin described important features of preparing the anatomical structures of the recipient for successful replacement of an inoperative trachea with a transplant, followed by vascular stitches on the selected recipient arteries and the speedy restoration of blood circulation.

Largely thanks to Dydykin, the Russian surgeons were the first to successfully conduct a simultaneous transplantation of a live trachea as part of a thyrotracheal complex (two donor organs transplanted together, the trachea and the thyroid gland located in front of it). The patient does not need a second (andforeign) thyroid gland, yet it has vessels common with the trachea and convenient enough for surgical surgeons to stitch them and thus restore blood circulation in the transplant. The thyrotracheal complex is transferred to the patient‘s body immediately after removal of the patient‘s narrowed and pathologically irreversibly changed trachea. The ends are orthotopicallystitched, and then vascular stitches connect the left and right lower thyroid arteries to the recipient's brachiocephalic arterial trunk. For the outflow of venous blood from the trachea, the lower thyroid graft vein is stitched into the lateral surface of the recipient's left brachiocephalic vein. In this case, the tribute is to be paid to Macchiarini, who was the first to justify the possibility of transplanting a donor thyrotracheal complex for successful replacement of the trachea (in 1994 in an experiment on pigs). He discussedthe advantages and disadvantages of various methods of restoring blood circulation in an allogeneic tracheal transplant and demonstrated the importance of ensuring not only the arterial blood flow to the trachea but also an adequate venous outflow. Macchiarini had no experience in transplantation of a donor trachea to a person but performed complex tracheoplastic operations. For example, he operated on more than 20 children under the age of 1,5 because of congenital contractions and softening of the trachea, a complex pathology. He successfully performed sliding tracheoplasty and circular resection of the trachea with good clinical results.

Nikolai Olegovich Milanov (1950–2014) plastic surgeon, academician of RAMS, doctor of medical sciences, professor, laureate of the USSR State Prize and the Russian Government Prize, Honored Scientist of the Russian Federation

Vladimir Dmitrievich Parshin, Doctor of Medicine, Professor, Head of the Department of Surgery of the Lungs and Mediastinum, Petrovsky National Research Centre of Surgery, RAMS. Secretary of the Thoracic Surgery Problem Commission of the Surgery Council of RAMS. President of the Moscow Society of Thoracic Surgeons

Thus, successful simultaneous transplantation of a viable donor trachea with the thyroid gland and resumed blood circulation in this donor organ through the vessels of the thyroid gland was performed for the first time on October 19, 2006 at *Petrovsky National Research Centre of Surgery* of the Russian Academy of Medical Sciences. The patient was a 36-year-old male with subtotal stenosis (almost complete narrowing) of the trachea. The 40-year- old male donor‘s brain death occurred because of severe traumatic brain injury. Outstanding surgeons Milanov and Parshin performed the operation using microsurgical equipment. The donor trachea was installed from the level of the first interchondral space to the level of the tracheobronchial angles. The patient received immunosuppressive therapy, the same as with a lung transplant. 5-year observation showed that the blood supply to the tracheal wall was preserved and there were no signs of damage to it. The transplant fully performed all the functions of the trachea, which allows speaking to some extent (the patient is still taking drugs that suppress the immune system) about curing. The operation was extensive and had a high degree of complexity; the wait for the donor trachea amounted to 254 days. After the transplant, the patient developed problems with immune rejection and endocrine disorders - the work of his own, previously healthy, thyroid gland was disrupted. Despite all the difficulties, theforeign trachea continued to function normally, and the condition of the lungs was also normal. This patient was able to completely restore the air-conducting function of the trachea. Within a year after transplantation, it was possible to reduce the dose of cyclosporin A to 200 mg/day and methylprednisolone to 2 mg/day, but the dose of mycophenolatemofetil was left unchanged and quite large - 2 g/day. Two years after transplantation, the donor trachea narrowed in the lower part due to external compression; it was necessary to temporarily install a stent in the lumen of the trachea to restore breathing (14).

Head of the research team, Pierre *Delaere* (Head of the research team of the Clinic for Experimental Otorhinolaryngology at the University of Leuven (Belgium), tried to solve the main problem in tracheal transplantation: how to

connect the vascular system of a foreign organ in the new body with the existing small blood vessels around the trachea (9,10). As noted above, due to the small diameter, arterial branches feeding the trachea are almost impossible to stitch with the vessels of the donor organ even using microsurgical technique. If something can be stitched together, it is still insufficient for the blood flow to be intense. Before transplantation, *Delaere* decided to create a new vascular system in the donor trachea from the recipient's own vessels. The system is created with the expectation of subsequent connection to large vessels and the restoration of full blood supply to the trachea. The small vessels surrounding the new organ can later grow independently into the trachea and improve blood circulation in it. Belgian scientists, using a method similar to the method of Klaus Herberhold, maximally freed the cartilaginous half rings from the surrounding tissues, and removed the membranous back wall of the trachea. Then, the remaining part of the trachea — the cartilaginous half rings, the submucosal layer, and the mucous membrane with ciliated epithelium from the donor — was implanted under the skin in the forearm, additionally surrounded by the soft tissues and fascia to form a flap. A feeding pedicle was formed from the fascia, the radial artery and the vein of the arm. In contrast to the prototype (Rose‘s operation with colleagues) a more convenient and less dangerous place was chosen here for the preliminary preparation of the transplant – the forearm.

The foreign trachea was transplanted under the skin of the recipient's forearm where it was allowed to grow for three months. All this time, the patient received immunosuppressants. This transplant preparation was called revascularization – re-formation of one's own vessels in the trachea. After complete restoration of blood circulation in the foreign mucous membrane, part of it was replaced by the mucous membrane from the recipient's oral cavity. Over the next month, the patient‘s own mucosa was implanted in a foreign trachea living in his arm, and the entire prepared complex of foreign and own tissues was ready to create a new trachea for the patient. After organ transplantation, immunosuppressive therapy was gradually weakened; the patient‘s own immune system was gradually activated and began to attack foreign cells and tissues, and the foreign mucosa of the donor trachea was

gradually replaced by the recipient's own mucosa. It turned out that during such operation, the patient‘s body did not reject the foreign cartilage of which the hyaline half rings of the trachea consist. Specialists are aware of this phenomenon: due to its specific structure, cartilage does not have its own blood vessels, and cartilage cells are enclosed in a dense ECMconsisting of cartilage fibrils which are not immunogenic and do not let immunity effect cells and some large protein molecules through. About a year after transplantation, immunosuppressants were completely withdrawn.

An interesting fact is noteworthy – all the cartilage tissue of the hyaline half rings of the trachea belonged to the donor (despite the fact that suppression of immunity was ceased) and provided mechanical rigidity of this section of the respiratory tract, almost the same as that of a normal trachea. The question remains regarding the cellular composition of the perichondrium, which requires additional research.

The Leuven team successfully operated on six patients using this technology; none of them showed rejection even years after the cancellation of immunosuppressive drugs. Belgian scientists also drew attention to the possibility of using or mobilizingown organs and tissues of patients to grow complete new organs on the basis of donor structures. The idea of using the patient‘s own organs as bioreactors to grow their own tissues is very popular

– it can be said that new horizons opened in the development of prefabrication. Not only do connective tissue and new vessels grow into the temporarily planted organ, but scientists also assemble on the spot or complete the organ with the necessary own tissues – replacement of foreign tissues by full-fledged own ones during regeneration by transplant is managed in a way.

The first operation using this technique was performed on 54-year-old Linda De Croock whose trachea was badly damaged after a car accident. The lumen of the trachea narrowed drastically, and life-threatening breathing problems arose. To prevent the woman from suffocating, short tube expanders (metal stents) were installed in the lumen of the trachea. The metal frame, covered with inert silicone, provided air permeability but caused considerable discomfort to the patient – for several years Linda suffered from neck pain, breathing problems, and periodical severe cough, and lost her sense of smell. To reduce inflammation around the stents, the patient had to take anti-inflammatory drugs, including immunosuppressants that were ruining her health. Growing a new trachea in the forearm and preparing for organ replacement took 10 months, yet in the end, the patient was able to return to the normal and healthy life without taking medication and without stents. Her fever, tormenting cough and exhausting pain disappeared, and her sense of smell returned.

In 1980, Professor Hans Anderl (*Department of Plastic and Reconstructive Surgery, the University of Innsbruck)*

managed to use a displaced flap to repair a tracheal defect in a female after a car accident. Namely, bilateral deltopectoral skin flaps were used, from which a new breathing tube of an unusual structure was formed during the operation. A deltopectoral flap is a type of arterial skin flap which feeds on the perforating branches of the internal artery of the mammary gland and is located above the deltoid and pectoral muscles. To be able to maintain its shape and not to collapseduring breathing, the newly formed tube was reinforced on the outside with strips of rib cartilage specially cut from the same patient and given a horseshoeshape like the hyaline cartilage of the trachea. The operation allowed to restore air permeability and ensured a sufficiently high quality of life after treatment. The patient is still alive, the period of observation exceeds 30 years. The cartilage constructions were periodically resorbed, so the patient was twice subjected to correction operations fixing the walls of thisneotrachea. Instead of costal cartilage, the respiratory tube was subsequently strengthened with artificial material - horseshoe-shaped Gore- texhalf rings. Gore-texis synthetic material manufactured by W. L. Gore & Associates whichhas been manufacturing vascular prostheses and other implants from polytetrafluoroethylene since 1972. This is an inert and biocompatible material with antimicrobial properties, and after implantation in a living organism, it is

able to long maintain constant volume and shape without undergoing either degradation or resorption. French thoracic and plastic surgeons continued the work begun by Hans Anderl and in 2004 developed their modification of the technology for creating a neotrachea from own skin flaps of patients. Operated patients with a new trachea were monitored 6 years after the operation. This tracheal restoration technique is primarily suitable for cancer patients with a lesion of at least 50% of the trachea length, since it does not require either temporary or permanent suppression of immunity, and artificial support for the lumen of the new trachea with stents is not required. Tracheal tumors are fatal diseases leading to suffocation (asphyxia) in the short term (39).

Plastic surgeons (*Philippe Dartevelle, Professor of Thoracic and Cardiovascular Surgery of Paris Sud-University, Head of the Department of Thoracic, Vascular Surgery and Cardiopulmonary Transplantation at Marie Lannelongue Hospital)* create a rectangular skin flap with feeding vessels and turn it (epidermis inside) around a special silicone tube. The diameter of the tube corresponds to the diameter of the normal lumen of the trachea. To impart structural rigidity, 6–7 strips (cut from own costal hyaline cartilage) are inserted into the skintube in a special way between the skin and subcutaneous tissue (see the figure below).
After the healing of the skin tube and the fusion of the strips of hyaline cartilage, the neotrachea is transplanted instead of the removed unsuitable own trachea (*Frederic Kolb, professor, chief plastic surgeon at the GustaveRous-*

*sy Institute in Villejuif).*To build a new trachea, surgeons used a flap of patient‘s own skin on the forearm., and using the microsurgical technique, the feeding pedicle of this design is stitched with the vessels surrounding the trachea to quickly restore blood circulation in the neotrachea. The operation to transplant such artificially created organ into the correct position takes about 12 hours (11). The operation took place six years ago, when the patient was 35; breathing was impaired due to the destruction of the trachea, and a fistula between the trachea and the esophagus complicated the situation. Today, this patient breathes freely, eats and lives a normal life.

In total, since 2004, the trachea was restored in 12 patients with tracheal resections of 7 to 12 cm (an average of 11 cm). In all patients, tubes for artificial air supply to the lungs were removed one day after the operation. 8 out of 12 patients survived; the average period of their neotracheas functioning was 36 months(2 to 94 months from the moment of surgery), without the development of life-threatening inflammatory lung lesions usually accompanied by diffuse infiltration and severe hypoxemia. Two patients died from a pulmonary infection. None of the patients experienced collapse (subsidence) of a new part of the airways, which was monitored by endoscopy, dynamic computed tomography, and, indirectly, spirometry. The obtained result allowed the authors to consider such a transplant a long-term replacement of part of the trachea.

French surgeons rightly consider the lack of ciliary cells and ciliary transport inside a new breathing tube (which does not allow patients to effectively get rid of sputum and tracheobronchial secretion) the main problem in need of a solution. Patients with cystic pulmonary fibrosis face a similar problem. A search is

underway for a technical solution that would restore a full mucous membrane characteristic of the airways of this location in the similar trachea design.

6. Organ cultivation in *in vivo* bioreactors.

In general, the idea of the first prototype of a bioreactor in a living organism belongs to Wayne A. Morrison (Professor of the University of Melbourne). He created a model of a mobilized arteriovenous shunt that was inserted into a chamber implanted in the animal's body to partially distinguish the shunt and surrounding tissues from the body's internal environment. Clearly, this technical solution has a completely different meaning.

The ideas of regenerative surgery, the possibility of restoration and manifestation of regenerative ability in mammals, as well as the ability to create special conditions for regeneration by mammals and humans, including the heart muscle, brain, cranial vault, retina and lens of the eye, etc. were born and developed in the USSR, largely ahead of time. A powerful foundation was laid for further development of regenerative technologies in Russia.

To stimulate regeneration, scientists used transplantation in particular, yet with a different goal. Therefore, the Soviet professor Lev Vladimirovich Polezhaev proposed the concept of regeneration induction (31). If the individual is unable to independently restore the normal structure of a particular organ, an external effect is exerted on the regeneration process. Instead of the lost part of the organ, a regeneration inductor is introduced into the damage zone — these can be transplanted shredded tissue or organ fragments with or without living cells. In fact, specially prepared grafts-inductors are transferred to the trauma zone. A new part of the organ is formed instead of the lost one and is almost identical to it in structure. Thus, the integrity of a number of organs can be restored: the cranial vault in mammals (Polezhaev) and humans (Sinitsa), muscles (Studitsky) and retina (Lopashov) in laboratory rodents (3). Allostatic transplant regeneration was partially studied using transplantation of nerve cords, etc. However, such newly formed, regenerated parts of organs are called regenerates, but not bioartificial organs. That is, this new strategy proposed by Macchiarini actually does not relate to tissue engineering, and, moreover, does not apply to the cultivation of bioartificial organs; this is obvious even despite the absence of generally accepted definitions of tissue engineering and bioartificial organs.

The author of the present research developed a method for the conservative treatment of finger injuries in children of 1-12 years old (19). This method helps children with traumatic defects of the tip of the nail phalanx completely regenerate the lost part. A full recovery is observed after six months or a year, and no traces of the trauma are left.

The damaged finger is enclosed for 16-24 days in a special isolation device filled with a periodically replaced special sterile solution. The device is attached to the hand and slightly limits the range of motion of the child, while the wound is isolated from the air. The treatment of the fingers is painless; no anesthesia is required.

Regenerating epidermis is necessary for initiating the regeneration of a limb or tail in animals capable of epimorphic regeneration. If the limb amputation wound of a newt is covered with a full-layer skin flap, regeneration will not occur. Similarly, in children, the natural regeneration of the fingertips is completely inhibited if the wound is closed surgically by stitching the edges of the wound or moving the skin flaps (20). Therefore, an isolation device with an aqueous medium, like long-term irremovable dressings, creates a special local microenvironment around the wound, yet more favorable and more reliably preventing injury and infection.

Each stage of the regeneration process in mammals can be strongly influenced by the local environment which is a decisive factor of success. Attraction of tissue-specific progenitor cells can be suppressed by an inflammatory response. Growth factors in the lesion zone can play an important role in maintaining or inhibiting cell proliferation. The local ECM is a necessary foundation at the early stages of regeneration since it is able to provide a conductive medium for cells, including bone marrow stem cells circulating in peripheral blood, and provide early

orientation of regenerating tissue. Therefore, the method of regenerative therapy of injuries in an aqueous environment can form the basis of more complex – biotechnological – methods of treatment, effectively modifying the natural course of recovery processes.

Fingertips in rats and mice in the postnatal period of development do not regenerate, yet in the prenatal period this is possible. Using the example of epimorphic finger regeneration in mouse embryos, the role of the Msx family of homeobox genes involved in this reaction was revealed. In all four-legged vertebrates, Msx1 and Msx2 are coexpressed in the apical mesenchyme during the entire period of limb formation. In animals that repair their limbs, for example, newts and salamanders, Msx genes are activated during regeneration and inactive during redifferentiation. During restoration of the fingertip in the mouse embryo, both Msx1 and Msx2 are expressed in regenerative connective tissue, however, if the volume of amputated tissues is increased, there is no expression zone for these genes and restitution is impossible. It is believed that regeneration of the fingertip depends on the cells of the connective tissue underlying the claw, where expression of the Msx genes is detected. The importance of this cell population (undifferentiated mesenchymal cells) for regeneration of the formed finger is also confirmed by histological observations that note the flow of fibroblast-like cells to the regenerating fingertip. It was noted that the cells of the growth zone of the nail plate do not participate in the regeneration of the finger (15, 34, 40).

In prenatal ontogenesis, the proximal parts of the limbs are first to lose the ability to restitution. It is likely that with the growth of the child, the number of undifferentiated mesenchymal cells in cell populations of the connective tissue of the nail phalanx decreases, and one of the basic principles of regeneration is that a cellular source of new tissue is required. A deeper understanding of the possibilities of enhancing natural processes with the necessary progenitor cells grown *in vitro* and subsequently implanted in the

desired areas is a way to improve the regenerative methods of treating injuries.

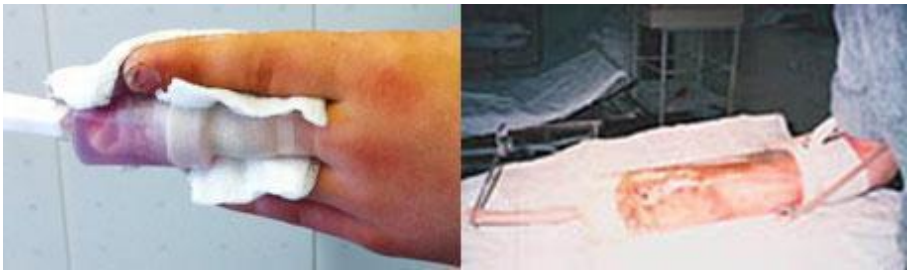
In an isotonic sterile solution in the damage zone, post-traumatic inflammation and the number of inflammatory response effectors are reduced. This can activate a population of progenitor mesenchymal cells that realize their morphogenetic potentials. Despite the morphological characteristics of the restoration of the fingertip in children, this process is not attributed to epimorphic regeneration. Unlike epimorphosis, with this type of regeneration, characteristic interconnected morphological structures are not formed - the apical thickening of the epidermis (apical epidermal cap) and blastema (accumulation of undifferentiated mesenchym-like cells in the distal part of the stump). Only one, the last segment of the finger is restored. Regeneration of the fingertip cannot be attributed to scarring either – there is a theory that mammals are not able to spend the considerable time required for epimorphic regeneration of structures, and that scarring, being a faster process, is a more effective replacement for restitution.

Like the fingertips in children (organotypically) the finger, metacarpal and carpal torus of hedgehogs and dogs is restored. The torus is a pillow-like thickening of the skin on the paws and is a rather complex organ. The basis of its skin (corium tori) forms long papillae that connect to the epidermal layer of the torus. Its subcutaneous basis is well expressed in the form of an elastic fat pad pierced by collagen and elastic fibers. The finger torus also contains sweat glands. If in an experiment, a dog‘s torus is removed without affecting the surrounding skin, three months later complete recovery is observed with almost no morphological differences from intact paws. Full healing of the torus is associated with constant mechanical stress and frequent damage; partial healing and formation of coarse scars could adversely affect the survival of the species.

In children, phalanx lesions are most common at the age of 5.5-3.8. The described ability to recover (under favorable conditions) is probably a species trait fixed in evolution – an adaptation to frequent damage to the most vulnerable structures (fingertips). Moreover, the ability to fully restore the finger appears at the age when these injuries are most common.
Thus, the proposed technology for the conservative treatment of children aged 1-12 (the period of postnatal development) with traumatic defects of the fingertips effectively stimulates the regenerative growth of the distal part of the nail phalanx in an aqueous environment. This is an example of a possible direction for the development of regenerative medicine and can be recommended for implementation in practical healthcare (19).

Since 2003, our laboratory used cultivated cells and a cell-free matrix of the connective tissue of the skin to restore the skin in a special way (Patent No. 2234312, priority from 2003). Restoring an organ using cell transplantation and an fiber _skeleton‘ is far from easy – there are many unresolved technical problems in this area, and special conditions are required of which Macchiarini was not fully aware since he had never been engaged in regeneration in a biological sense. For instance, everyone who is familiar with reparative regeneration in mammals is aware of excessive post-traumatic inflammatory reaction which creates a serious local change in many physicochemical

parameters of the internal environment in the damaged area. Mostly inflammatory effector cells, fibroblasts and cells of newly formed vessels can _work‘ there, so in warm-blooded animals (including humans), mainly young connective tissue is actively developing in the injury zone. Usually, young connective tissue also develops instead of a full restoration of organs; this is called substitution, and its final stage is defective regeneration (scarring). During scarring, densification of the newly formed connective tissue (fibrosis, sclerosis) and contraction of the surrounding healthy tissues occurs, leading to deformation of neighboring tissues and organs of varying severity.



Portable devices of our design with special liquid media for controlling tissue regeneration of fingers and hands in special conditions

Therefore, in order to build a new organ or part of it directly in the body of living individuals, in our experiments we actively intervene in the restoration processes and surround tissue-engineering structures with a certain barrier - circulating nutrient media with special properties. They weaken inflammatory reactions and scarring, and more favorable conditions are created for the normal secondary development of an organ or tissues. The special devices we use are called regeneratrons. Regeneration is monitored by the researcher according to biochemical and physicochemical parameters by constant analysis and changes in the physical properties and composition of the _intermediary media‘.

When using a managed aqueous media strategy, new technical possibilities to influence regeneration appear. A bioreactor is an essential component of the classical triad of tissue engineering technology (cells, scaffold,

bioreactor). Regeneratrons – *in vivo*bioreactors integrated with a living organism – are the latest generation of such devices and are still rarely used in clinical practice. The present research aimed at developing, building and testing such device on laboratory mammals to build tissue-engineering structures directly on the wound surface. To create a controlled aqueous environment around the damaged areas of the body, specially designed chambers were used, hermetically connected to the skin surface and bathing the wound in solutions. The bioreactor (integrated with the body of an animal) with the function of pneumoacoustic spraying of serum-free medium includes an isolation chamber and a special atomizer with a flexible pipe for supplying fluid. The tissue-

engineering structures are located on the wound surface, air-water mixture in the form of an aerosol cloud is supplied through nozzles in the container wall, and the temperature of this mixture is maintained at a constant level, which ensures adequate cellular respiration and nutrition during the interaction of a living organism and tissue-engineering structures. The gas composition is easily adjustable; furthermore, gas transmitters may be added. Improving the design of medical isolation cells allowed replacing saline isotonic solutions with culture media, as well as developing and introducing into clinical practice new methods for the conservative treatment of post-traumatic partial defects of the nail phalanges not only in children but also in adult patients. Regenerating fingertips, despite signs of an atypical structure, successfully closed the defect. The bioreactor allows effectively increasing cell mass inside tissue- engineering structures located on the wound surface at the initial stages of engraftment. The thickness of scaffolds can be increased; under such conditions, living cells with high synthetic activity are located throughout the thickness. The present research demonstrates that a new bioreactor integrated with the body of an animal has been created, tested and has already shown its functionality. This is an important step towards improving bioreactor technology for tissue engineering.

Conclusions

Today, research in the development of bioartificial organs is carried out for almost every organ existing in the human body. The most exciting are the first achievements in the field of clinical use of bioartificial organs.

Biofabrication of some hollow organs (bladder, ureter, vagina, etc.) and their subsequent orthotopic transplantation demonstrate an effective restoration of structure and functions. Long-term follow-up of patients does not reveal any serious complications and regression of organs requiring repeated interventions. The quality of tissue- engineering structures of bioartificial organs is gradually improving. In some cases, it is possible to achieve complete rehabilitation of patients. However, the limited clinical experience of using the most successful examples of new organs created this way and the obvious imperfection of their production technology, the uncertainty of the engraftment stages, regeneration mechanisms and subsequent remodeling, determine the need for additional experimental and clinical studies.

Using the trachea as an example allows stating that hasty implementation, inadequate development and inadequate preclinical testing of organs, gaps in knowledge of regeneration and of its research history can be deadly for patients. It must be understood that invasive treatment methods – which in some cases mean rapidly developing innovative reconstructive surgery – successfully compete with technologies of bioartificial organs.

Today, the desire to carry out a simultaneous operation prevails, but, in our opinion, cultivating new bioartificial organs or repairing organs with pronounced irreversible pathomorphological changes is most effective in a controlled local regeneration environment. In a broad view, all the achievements of tissue engineering can be applied, in one form or another, for growing living

structures inside bioreactors integrated with the body of the recipient. An important principle for constructing new biological structures inside such bioreactors, in our opinion,

is the gradualness and sequence of building up bioartificial organs and tissues directly at the border with the recipient's body tissues. With this approach, tissue bioengineering is combined with an important known method of regeneration – regeneration by transplant or scaffold. The tissue-engineering structure, during remodeling, turns into a full-fledged organ, and the regeneration of vessels and of the nervous system through this transplant allows effectively integrating the construct into a living organism and carrying out a full repair of a living object and of its pathologically altered organs. Narturally, the principle of gradual biofabrication on a living object is primarily suitable for organs consisting mostly of connective tissues, such as bone and skin.

We are optimistic about the future: accurate bio-mapping of organs, analysis of signaling pathways, results of studies of cell populations and of structural units of organs and tissues, expanding the list of widely available cytokines, differentiation inducers, substances of amorphous substances and fibers, and improving bioreactor technologies will undoubtedly accelerate development of bioartificial organs. Integration of various specialists anduse of interdisciplinary approaches will undoubtedly contribute to the creation of perfect bioartificial human organs.

References

[1] Atala A. New methods of bladder augmentation. BJU Int. 2000 May;85Suppl 3:24-34; discussion 36. doi: 10.1111/j.1464-410x.2000.tb16938.x.PMID: 11954194

[2] Atala A., Bauer S. B., Soker S., Yoo J. J., &Retik A. B. (2006). Tissue-engineered autologous bladders for patients needing cystoplasty. The Lancet, **367**(9518), 1241-1246. doi:10.1016/S0140-6736(06)68438-9

[3] Бабаева, Анна Георгиевна.Регенерация: факты и перспективы / А. Г. Бабаева; Российская акад. мед. наук, ГУ НИИ морфологии человека РАМН. - Москва : Изд-во РАМН, 2009. - 333, [1] с.: ил.; 21 см.; ISBN 978-5-7901-0085-7 Babaeva, A.G. Regeneration: facts and prospects. - Moscow: RAMS, 2009. - 333, [1].

[4] Baptista PM, Orlando G, Mirmalek-Sani SH, Siddiqui M, Atala A, Soker S. Whole organ decellularization - a tool for bioscaffold fabrication and organ bioengineering. ConfProc IEEE Eng Med Biol Soc. 2009; 2009:6526-9. doi: 10.1109/IEMBS.2009.5333145. PMID: 19964173

[5] Birchall M., Macchiarini P. Airway transplantation: a debate worth having? Transplantation 2008; 85: 1075–80.

[6] Calle E. A., Ghaedi M., Sundaram S., Sivarapatna A., Tseng M. K., Niklason L. E. Strategies for Whole Lung Tissue Engineering IEEE Trans Biomed Eng. Author manuscript; available in PMC 2015 May 1. Published in final edited form as: IEEE Trans Biomed Eng. 2014 May; 61(5): 1482–1496. Published online 2014 Mar 28. doi: 10.1109/TBME.2014.2314261

[7] Crowley C, Birchall M, Seifalian A.M.Trachea transplantation: from laboratory

to patient.J Tis- sue Eng Regen Med. 2015 Apr;9(4):357-67. doi: 10.1002/term.1847.
[8] Chen Y, Geerts S, Jaramillo M, Uygun BE. Preparation of Decellularized Liver Scaffolds and Recellularized Liver Grafts. Methods Mol Biol. 2018; 1577:255-270. doi: 10.1007/7651_2017_56. PMID: 28735385
[9] Delaere P., Van Raemdonck D. Tracheal replacement J Thorac Dis. 2016 Mar; 8(Suppl 2):
S186–S196.doi: 10.3978/j.issn.2072-1439.2016.01.85
[10] Delaere P.R., Van Raemdonck D. The trachea: The first tissue-engineered organ? J Thorac. Car- diovasc Surg. 2014; p. 519-5)
[11] Fabre D, Singhal S, De Montpreville V, Decante B, Mussot S, Chataigner O, Mercier O, Kolb F, Dartevelle PG, Fadel E. Composite cervical skin and cartilage flap provides a novel large airway substitute after long-segment tracheal resection. J ThoracCardiovasc Surg. 2009 Jul;138(1):32-9. doi: 10.1016/j.jtcvs.2008.11.071.
[12] Figliuzzi M., Bonandrini B., Remuzzi A. Decellularized kidney matrix as functional material for whole organ tissue engineering. J ApplBiomaterFunct Mater. 2017 Nov 10;15(4): e326-e333. doi: 10.5301/jabfm.5000393.
[13] Geerts S., Ozer S., Chris Chu, Fuchs B. C., Tanabe K. K., Yeh H., Uygun K. Exploring donor demographics effects on hepatocyte yield and viability: Results of whole human liver isolation from one center Technology (Singap World Sci) Author manuscript; available in PMC 2019 Aug
14. Published in final edited form as: Technology (Singap World Sci). 2019 Mar-Jun; 7(1-2): 1– 11. Published online 2019 Apr 26. doi: 10.1142/S2339547819500018
[14] Gudovskiĭ L M, Milanov N O, Parshin V D, Trofimov E I Long-term Results of Correction of Extensive Tracheal Defects by Means of Microsurgical Technologies/ ProblTuberkBolez- nLegk2006;(3):18-23.PMID: 16817553
[15] Han M, Yang X, Lee J, Allan CH, Muneoka K. Development and regeneration of the neonatal di- git tip in mice // Dev Biol.- 2008. – V. 315, N. 1. – p. 125-35. doi: 10.1016/j.ydbio.2007.12.025. Epub 2007 Dec 27.
[16] Hamilton N. J., Kanani M., Roebuck D. J., Hewitt R. J., Cetto R., Culme-Seymour E. J., Toll
E., Bates A. J., Comerford A. P., McLaren C. A., Butler C. R., Crowley C., McIntyre D., Sebire
N. J., Janes S. M., O'Callaghan C., Mason C., De Coppi P., Lowdell M. W., Elliott M.
J., Birchall M. A. Tissue- Engineered Tracheal Replacement in a Child: A 4- Year Follow- Up StudyAm J Transplant. 2015 Oct; 15(10): 2750–2757.Published online 2015 Jun
2. doi: 10.1111/ajt.13318
[17] Hollander A. P. Cell therapies and regenerative medicine - the dawn of a new age or more hype than hope? ClinTransl Med. 2012; 1: 12. Published online 2012 Jul 3. doi: 10.1186/2001-1326-1-
12
[18] .Iop L., Dal Sasso E., Menabò R., Di Lisa F., Gerosa G. The Rapidly Evolving Concept of Whole Heart Engineering.
Stem Cells Int. 2017; 2017:8920940. doi: 10.1155/2017/8920940. Epub 2017 Nov 9.
[19] Kovalev A.V., Gerasimov D.N., Ivanishchuk P.P., Lvov S. E. Recovery of

damaged nail phalanges of the fingers in children with the use of chamber-isolators with aqueous medium. Bulletin of Traumatology and Orthopedics. N.N. Priorov. 2008. No. 4. P. 56-60.
[20] Kovalev A.V., Ivanishchuk P.P. PARTIAL REGENERATION OF THE TAIL TIP OF NEW- BORN RATS IN A LIQUID MEDIUM Ontogenesis. 1999. T. 30. № 1. C. 47-50.
[21] Лиознер, Лев Давидович Регенерационные процессы и их изучение в СССР / Л. Д. Лиознер, А. Г. Бабаева, И. В. Маркелова. - М.: Изд-во МГУ, 1990. - 107,[1] с.: ил.; 21 см.; ISBN 5- 211-00924-XLiozner, L.D. BabaevaA.G., MarkelovaI. V. Regeneration processes and their study in the USSR. M.: Publishing House of Moscow State University, 1990.
[22]Langer R., Vacanti J. P. Tissue engineering. Science. 1993. May 14;260(5110):920-6
[23] Macchiarini P., Jungebluth P., Go T. et al. Clinical transplantation of a tissue-engineered airway.
Lancet 2008; 372(9655): 2023-30.
[24] Macchiarini P., Walles T., Biancosino C., Mertsching H. First human transplantation of a bioen- gineered airway tissue. J. Thorac. Cardiovasc. Surg. 2004; 128: 638–41.
[25] Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Cogan TA, Dodson A, Martorell J, Bellini S, Parnigotto PP, Dickinson SC, Hollander AP, Mantero S, Conconi MT, Birchall MA.Clinical transplantation of a tissue-engineered airway.Lancet. 2008 Dec 13;372(9655):2023- 30. doi: 10.1016/S0140-6736(08)61598-6. Epub 2008 Nov 18.PMID: 19022496
[26] Macchiarini scandal is a valuable lesson for the Karolinska Institute. Nature. 2016 Sep 8;537(7619):137. doi: 10.1038/537137a. PMID: 27604911
[27] Madariaga ML, Ott HC. Bioengineering kidneys for transplantation. SeminNephrol. 2014 Jul;34(4):384-93. doi: 10.1016/j.semnephrol.2014.06.005. Epub 2014 Jun 13. PMID: 25217267
[28] Medalie DA, Eming SA, Tompkins RG, Yarmush ML, Krueger GG, Morgan JR. Evaluation of human skin reconstituted from composite grafts of cultured keratinocytes and hu- man acellular dermis transplanted to athymic mice. J Invest Dermatol. 1996 Jul;107(1):121-7. doi: 10.1111/1523-1747.ep12298363. PMID: 8752850
[29] Pellegata A. F., Tedeschi A. M., DeCoppiP. Whole Organ Tissue Vascularization: Engineering the Tree to Develop the Fruits Front. Bioeng. Biotechnol., 14 May 2018 | https://doi.org/10.3389/fbioe.2018.00056
[30] Porzionato A., Stocco E., Barbon S., Grandi F., Macchi V., De Caro R. Tissue-Engineered Grafts from Human Decellularized Extracellular Matrices: A Systematic Review and Future Perspec- tives. Int J Mol Sci. 2018 Dec 18;19(12). pii: E4117. doi: 10.3390/ijms19124117.
[31] Polezhaev, L. V. Regeneration by induction. - Moscow: Medicine, 1977.
[32] Shafiee A., Atala A. Tissue Engineering: Toward a New Era of Medicine. Annu Rev Med. 2017 Jan 14; 68: 29-40. doi: 10.1146/annurev-med-102715-092331. Epub 2016 Sep 30.
[33] Siddiqi S., de Wit R., van der Heide S., Oosterwijk E., Ad Verhagen Aortic allografts: final destination?—a summary of clinical tracheal substitutes J Thorac Dis. 2018 Aug; 10(8): 5149– 5153.doi: 10.21037/jtd.2018.07.108
[34] Takeo M, Chou WC, Sun Q, Lee W, Rabbani P, Loomis C, Taketo MM, Ito M. Wnt activation in nail epithelium couples nail growth to digit regeneration //

Nature. – 2013. - V. 499, N. 7457. – p. 228-32. doi: 10.1038/nature12214. Epub 2013 Jun 12.
[35] Taylor D. A., Parikh R. B., Sampaio L. C. Bioengineering Hearts: Simple yet ComplexCurr Stem Cell Rep. 2017; 3(1): 35–44. Published online 2017 Feb 10. doi: 10.1007/s40778-017-0075-7
[36] The final verdict on Paolo Macchiarini: guilty of misconduct. The Lancet.Lancet. 2018 Jul 7;392(10141):2. doi: 10.1016/S0140-6736(18)31484-3. Epub 2018 Jul 5.
[37] Tsuchiya T, Sivarapatna A, Rocco K, Nanashima A, Nagayasu T, Niklason LE. Future prospects for tissue engineered lung transplantation: decellularization and recellularization-based whole lung regeneration. Organogenesis. 2014 Apr-Jun;10(2):196-207. doi: 10.4161/org.27846. Epub 2014 Jan 31. PMID: 24488093
[38] Tsukada H, Ernst A, Gangadharan S, Ashiku S, Garland R, Litmanovich D, DeCamp M.Tracheal replacement with a silicone-stented, fresh aortic allograft in sheep.Ann Thorac Surg. 2010 Jan;89(1):253-8. doi: 10.1016/j.athoracsur.2009.09.005.PMID: 20103247
[39] Virk J. S, Zhang H., Nouraei R., Sandhu G. Prosthetic reconstruction of the trachea: A historical perspectiveWorld J Clin Cases. 2017 Apr 16; 5(4): 128–133.doi: 10.12998/wjcc.v5.i4.128
[40] Wu Y, Wang K, Karapetyan A, Fernando WA, Simkin J, Han M, Rugg EL, Muneoka K. Connec- tive tissue fibroblast properties are position-dependent during mouse digit tip regeneration // PLoS One. – 2013. – V. 8, N. 1. – doi: 10.1371/journal.pone.0054764. Epub 2013 Jan 18.
[41] Wurtz A., Hysi I., Kipnis E., Zawadzki C., Hubert T., Jashari R., Copin M.-C., Jude B. Tracheal reconstruction with a composite graft: fascial flap-wrapped allogenic aorta with external carti- lage-ring support Interact CardiovascThorac Surg. 2013 Jan; 16(1): 37–43.Published online 2012 Oct 9. doi: 10.1093/icvts/ivs422
[42] Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, *5*(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938