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Russian Federation
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Russian Federation**

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Dr. Paolo Macchiarini

Tracheal Transplantation

Clinical Trial Protocol

in "Molecular and Cellular Biology, Biotechnology, Regenerative Medicine"
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Budgetary Educational Institution of Higher Professional Education "Kuban State Medical
University," the Ministry of Health and Social Development of the Russian Federation and
the leading scientist Paolo Macchiarini performing scientific research for the time period
October 19, 2011 to December 31, 2013

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1.0 Clinical doctors - researchers

1.1 Leading researcher: Dr. Paolo Macchiarini, Karolinska Institutet, Stockholm, Sweden.

1.2 Clinical research coordinator: Professor Vladimir Porkhanov A., chief physician
GBUZ "Krasnodar Regional Clinical Hospital No. 1, n.a. S.V. Ochapovsky Department of
Health Care Krasnodar Krai.

1.3 Other researchers: Ph.D. Polyakov IS, Ph.D. IA Pashkov, Gilewicz IV, Fedorenko TV.

1.4 Research centers / clinics:

Clinics: State budget institution of Higher Professional Education "Kuban State Medical University" Ministry of Health and Social Development Federation Krasnodar Regional Clinical Hospital No. 1, n.a. S.V. Ochapovsky Department of Health Care Krasnodar Krai.

1.5 Funding: Ministry of Health and Social Development, The Russian Federation, The Russian Ministry of Education and Science.

2.0 Monitoring system

This protocol is designed as a request for permission to transplant trachea as an intraoperative solution for obstructive tracheal tumors and other conditions requiring replacement of the native trachea (table 1). The procedure involves the use of bioengineered synthetic scaffold seeded with autologous mononuclear cells, which is considered to be the only treatment option in some patients. Lead researcher, Dr. Paolo Macchiarini, will oversee the process with the assistance of a team of doctors and researchers who, together with the funding organization, will be responsible for monitoring of the patients before, during and after the procedure. These data are recorded in accordance to the requirements adopted for individual registration cards, which can be individually reviewed by an independent data monitoring committee or similar committee.

3.0 Introduction / brief overview

Tracheal transplantation is the only therapeutic alternative when endoscopic and other examinations shows that localization and extension of the obstruction (approximately 6 cm or more than 50% of the total length of the airway) make it impossible to perform surgery to remove the abnormal segment with adequate remaining length of healthy airway (table. 1). In the interim, patients can get temporary relief by endotracheal curettage and / or by inserting a T-tube into the trachea to maintain an open airway, but without surgical transplantation the disease will usually lead to death of the patient.

Table 1 - Indications for airway transplantation

Type of Disease	Rationale	Contraindications to Transplantation
Primary malignant tracheal tumors (benign and malignant)	Extension of the affected area beyond the limits for standard trachea resectability *	The presence of systemic metastases and mediastinal lymph nodes (malignant tumors); Conventional functional and psychological contraindications
Tracheal and esophageal fistula	Tracheo-esophageal defect exceeding the limits for standard trachea resectability	Malignant neoplasm
Tracheal stenosis	Extension of the affected area beyond the limits for standard resectability	Conventional functional and psychological contraindications
Tracheobronchial malacia (primary or secondary)	Extension of the defect area beyond the limits for standard trachea resectability treatment (luminal or stenting dilatation)	Conventional functional and psychological contraindications

*(6 cm of the entire length of the respiratory tract)

The proposed protocol involves replacement of the trachea of terminal patients by transplantation of bioengineered synthetic skeleton, seeded with autologous mononuclear cells.

3.1 Special training / experience

In addition to surgical methods for trachea transplantation the protocol requires the knowledge and the experience of preparation of autologous cells, as well as the cell seeding procedure of bioengineered synthetic scaffolds. Operation and appropriate training will be carried out by the lead researcher; cell preparation is monitored by a specialist from Karolinska Institutet, Stockholm, Sweden.

4.0 Approval of the clinical trial protocol

The protocol and the informed consent were approved by the Ethics Committee of the Kuban State Medical University at the meeting on December 21, 2011 (protocol no. 8). The new version of the protocol, the study booklet, voluntary informed consent and the patient registration card were approved by the Ethics Committee of the Kuban State Medical University at the meeting of February 15, 2012 (protocol no. 9) and by the local Ethics Committee at GBUZ "Krasnodar Regional Clinical Hospital No. 1, n.a. S.V. Ochapovsky Department of Health Care Krasnodar Krai" University on January 24, 2012 (protocol no. 45).

5.0 Brief description of previous research in humans

Clinical success of similar operations with transplantation of artificial tracheobronchial airway, performed at Karolinska University Hospital in Stockholm, Sweden, on June 6, 2011 and November 17, 2011, have shown that tracheobronchial transplantation using bioengineered nanocomposite scaffold and autologous mononuclear cells can offer the only chance for the recovery in incurable patients.¹

Previous studies have confirmed the ability of mononuclear cells to stimulate the migration of peripheral blood stem cells into the different tracheal layers and make them differentiate

¹Jungebluth P, Alici E, Baiguera S, et al. Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: proof-of-concept study. *Lancet* 2011 Dec 10; 378 (9808): 1997-2004.

into respiratory epithelium and cartilage cells.² In carrying out this transplantation procedure, we can not only fully remove the affected airway, but also give the patient an optimistic chance for cure and normal quality of life.

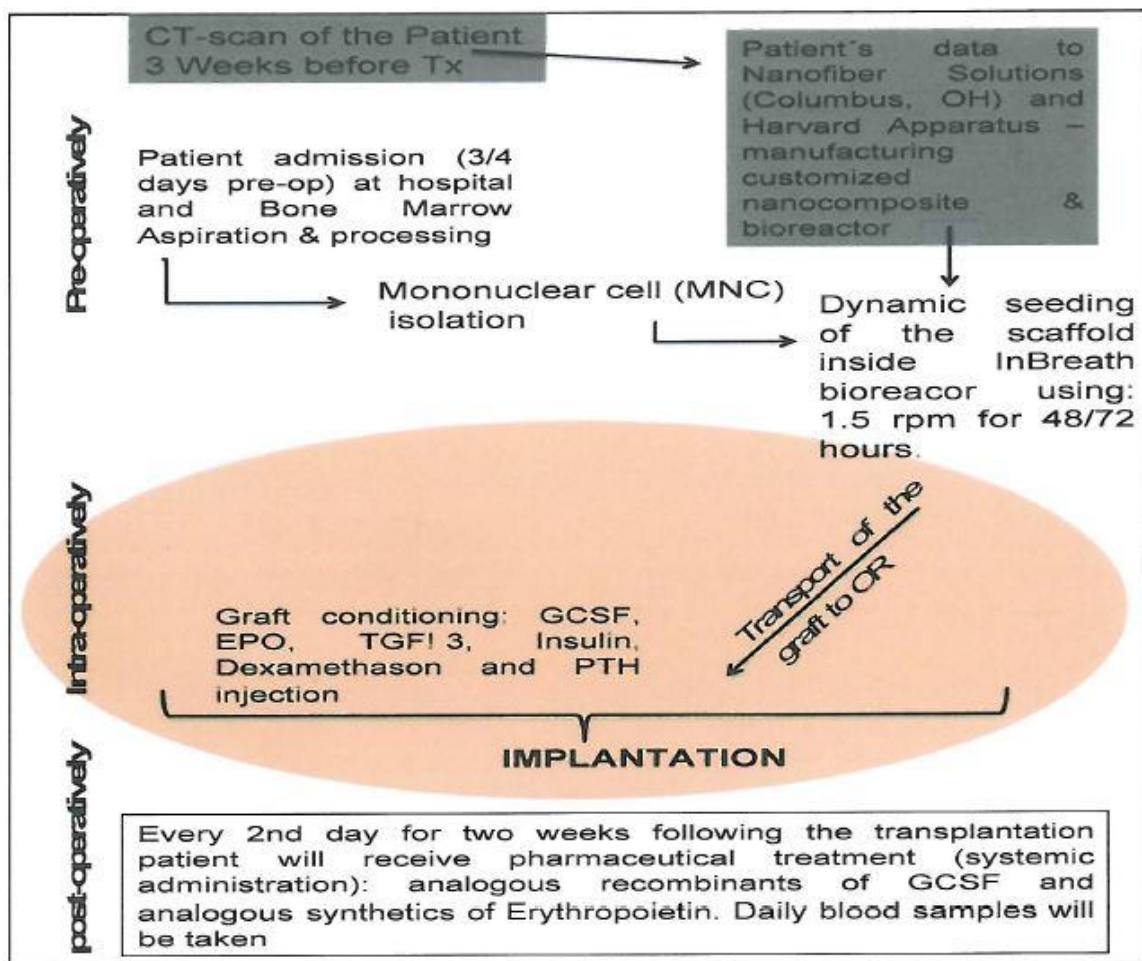


Fig. 1: Protocol for tracheal transplantation procedure, the first bioscaffold for transplant of artificial bioengineered trachea nanocomposite (PET bioscaffold).

Description

Preoperative procedures:

Computed tomography of the patient 3 weeks prior to transplantation

Patient data is delivered to the companies Nanofiber Solutions (Columbus, Ohio) and Harvard Apparatus (and other manufacturers) for fabrication of individual nanocomposite and bioreactor.

Admission of the patient to the hospital (3/4 days before surgery), bone marrow collection and processing

Allocation mononuclear cells

Dynamic seeding of bioscaffold inside the InBreath bioreactor at 1.5 rev / min for 48/72 hours.

²Macchiarini P, Jungebluth P, Go T, et al. Clinical transplantation of a tissue-engineered airway. Lancet 2008; 372,2023-3030.

Intraoperative procedures:

Preparation of graft: an injection of granulocyte colony stimulating factor G-CSF, erythropoietin, transforming growth factor beta-3, insulin, dexamethasone and parathyroid hormone.

Transportation of the transplant to the operating room

Transplantation

Postoperative procedures:

The patient receives the drugs (systemic application): recombinant G-CSF factor analogues and synthetic analogs of erythropoietin every second day for two weeks after transplantation transplant. Blood sampling is performed daily.

To date, two tracheal transplantations with bioengineered synthetic scaffolds have been successfully performed by Dr. Macchiarini together with colleagues from Karolinska Institutet in Stockholm, Sweden. In the first operation (June 2011) a nanocomposite bioengineered synthetic scaffold made of POSS-PCU (polyhedral oligomeric silsesquioxane) was used, while in the second operation (November 2011) a nanocomposite bioengineered synthetic scaffold made of PET (polyethylene terephthalate) was used. In both cases luminal ingrowth with healthy cells of respiratory epithelium was observed.

Fig. 2 shows the bronchoscopy results with respiratory epithelial cells after the operation in November 2011 with a bioengineered synthetic PET-nanocomposite bioscaffold was used, which is the same type of bioscaffold which is proposed in this protocol. The bronchoscopy and the pattern of stained cells show the presence of a normal mucosa in the bioscaffold at one week after transplantation.

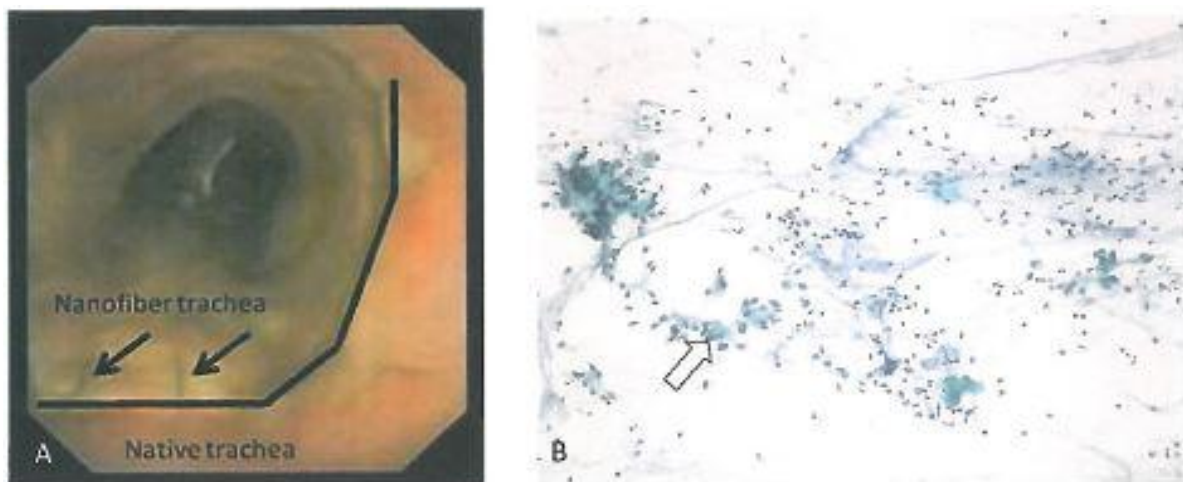


Fig. 2: **A.** Bronchoscopy of the PET-nanocomposite trachea one week after implantation (November 2011), showing presence of a normal mucosa on the bioscaffold. **B.** Ciliated respiratory epithelial

cells (white arrow) obtained from a brush biopsy from the center of the transplanted trachea one week after implantation. The fact that this biopsy was done almost immediately after transplantation, allows us to assume that the cells derive from the differentiated stem cells, and not from the spread of normal epithelial cells from the proximal or distal end of the transplant.

6.0 Rationale for not cancelling

In the previous research it has not been any negative effects or complications which would cause rejection of the proposed research plan (protocol).

7.0 The procedure for obtaining voluntary informed consent

Patient information is presented by informed consent (attached to the protocol). The form of informed consent for reference is issued to patients before the procedures and is necessary for inclusion in the study protocol. The patient is given the opportunity to familiarize themselves with the content of the informed consent in a separate room or bring it home to familiarize themselves with it, get answers to their questions and sign with the date and time of signing. All preparing of the patient for transplantation provided by the protocol starts after the agreement has been signed. Researchers register the date and time of its completion in the patient card.

8.0 General study plan

The tracheal transplant procedure will be performed sequentially, as shown in Fig. 1

"Protocol for tracheal transplantation procedure, transplantation for the first time applied for the with artificial bioengineered trachea nanocomposite (PET bioscaffold)".

Preoperative assessment will include the following procedures:

- Tomography of the neck and chest including a three-dimensional reconstruction of the respiratory tract.
- Rigid / Flexible fiberbronchoscopy.
- Evaluation of cardiac function (scanning with thallium during exercise).
- Assessment of respiratory function (spirometry).
- Analysis of blood, including blood coagulation factors.
- Evaluation of liver and kidney function.
- Immunogenic assess of peripheral blood sample for the determination of the phenotype HLA and serologic infections (HIV, syphilis, EBV, etc.).

- Evaluation of the patients hematopoietic stem cells baseline levels; approximately 30 ml of peripheral blood will be taken at admission for assessment basic level of hematopoietic stem cells, and a portion of this sample is frozen for further analyzes.
- Evaluation of endogenous baseline erythropoietin levels in the peripheral blood. In case of successful preoperative evaluation bone marrow samples will be selected for approximately 1-4 weeks prior to surgery.

Harvesting of mononuclear cells from bone marrow and seeding of the bioscaffold

[After 48 or 72 hours before transplantation depending on the necessary shape of bioscaffold (tubular or bifurcated shape)].

- The procedure will be performed under general anesthesia.
- Around 250-300 ml of bone marrow (BM) will be aspirated. BM will be passed to the Department of Hematology or other department to isolate mononuclear cells (MNCs).
- Cell medium DMEM (Dulbecco Modified Eagle's Medium) * + autologous serum (10%) + antibiotics.
- Peripheral blood sample (50 ml) will be aspirated with heparin (operating) and transferred to the laboratory for cell culture in compliance with good manufacturing practices. These MNCs will be isolated and frozen in liquid nitrogen.
- Bronchoscopy and bronchoalveolar lavage (BAL). It is necessary to keep the BAL (take away the supernatant, add PBS, pelleted by cell centrifugation and freeze).
- Bioreactor will be sterilized beforehand (locally with using plasma sterilization according to manufacturer).
- Synthetic bioscaffold will be sterilized with alcohol (ethanol) or by gamma-irradiation, and then incubated in cell medium in 2 hours before adding cells.
- Necessary materials: stitching and forceps, sterilized scissors
- Incubator.

- Seeding and growing the cells on a synthetic bioscaffold (scanning electron microscopy (SEM), microscopic studies of living cells, histology).

* - environment and their manufacturers may differ.

48 hours before transplantation:

The patients receive treatment to stimulate mobilization of cells by intravenous injection of recombinant granulocyte colony stimulating factor (Filgrastim, 10 µg/kg, not more than 30 million IU), and erythropoietin (EPO alpha or beta, not more than 40000 IU)^{3, 4, 5}.

The patient will receive full and accurate information through the informed consent form, orally and in writing, about the risks of the therapeutic procedure.

Cell preparation procedure

Bone marrow separation and further manipulation

72/48 hours before transplantation (depending on the desired shape of the bioscaffold: tubular or bifurcated) bone marrow samples (BM) will be selected by bilateral repeated aspiration from the iliac crest, general volume 250-300 ml. This procedure will be carried out under general anesthesia and lasts about 20 minutes.

Explanted aspirate will be transferred to the hematology unit (or to another department) to isolate mononuclear cells (MNCs). MNCs are obtained by Ficoll gradient separation, at density 1.077 g/ml. After separation the cells are washed three times with saline (with the addition of 5% human albumin) to remove the residual ficoll and left in a solution consisting solely of components approved for clinical use. The whole procedure will be carried out in a closed system (Sepax 3 Biosafe America Inc, Houston,

³Haas R, Murea S. The role of granulocyte colony-stimulating factor in mobilization and transplantation of peripheral blood progenitor and stem cells. *Cytokines Mol. Ther.* 1995; 1:249-70.

⁴Jia Wu, Warin R, Yu X, Epstein R, Noguchi CT. Erythropoietin signaling promotes cell progenitor transplanted survival. *FASEB J.* 2009; 23 (9): 3089-99.

⁵Brines M, Cerami A. Erythropoietin-mediated tissue protection: reducing collateral damage from the primary injury response. *J Intern Med.* 2008; 264 (5): 405-32.

Texas) to ensure sterility and a complete automatic process⁶. Isolated MNCs are transferred into a bag with 600 ml medium (Dulbecco's Modified Eagle Medium [DMEM 10% human albumin) and transported from operation at temperature 4°C to a laboratory working under the principles of GMP for filling synthetic bioscaffold (sterilized by ethanol or gamma radiation). After incubation (DMEM) for 2 hours the bioscaffold will be secured in the bioreactor. MNCs (medium DMEM) are seeded on the surface of the graft. Then corresponding medium and growth factors is added (10µg/cm² of recombinant human transforming growth factor β-3 (R & Systems, Minneapolis, Minnesota, United States), 10 nmol/l recombinant parathyroid hormone-related peptide (PeproTech), 100 nmol/l dexamethasone and 10 µg/ml insulin (Sigma-Aldrich, Dorset, United Kingdom). The bioreactor is placed into a incubator running with an initial rate of 1 cycle per minute for 18 hours, then the speed is gradually increased to 1.5 cycles per minute. After 48/72 hours the chamber is placed in a sterile container and carefully transferred to the operating room.

Isolated MNCs will be checked for the following indicators:

- The number of mononuclear cells (MNCs): the minimum amount of 2×10^6 cells/ml.
- Cell viability by fluorescence microscopy analysis (7-AAD): range 94-98%.
- Evaluation of mesenchymal progenitor cells CFU-F.
- Evaluation of hematopoietic progenitor cells CD34+.

Altogether 15×10^6 cells are placed in three separate cryo-vials and frozen in DMSO, in accordance with the standard procedure for quality control analysis. The sterile graft is then re-inoculated with

⁶Dal Pozzo S, Urbani S, Mazzanti B, et.al. High recovery of mesenchymal progenitor cells with non-density gradient separation of human bone marrow. *Cytotherapy*. 2010; 12 (5): 579-86.

cells in the operating room immediately before implantation (see Transplantation airway, page 14).

Artificial nanocomposite airway transplantation

The company Nanofiber Solutions (Doctor Jed Johnson, Columbus, Ohio) developed the graft trachea and tracheobronchial airways which is made of polyethylene terephthalate (PET). PET has been used successfully for more than 10 years for production of components to surgical implants and medical devices, ranging from non-absorbable suture thread including vascular transplants and orthopedic implants. Proposed polymer for the manufacture of a tracheal transplant is the biologically non-absorbable polyethylene terephthalate, that has been transformed into nanofibers (intermediate diameter of 350 nm), embedded in a semicircular spacer made of the material Dacron and forms a nanocomposite being completely biocompatible having the nanofiber structure of a natural trachea (Fig. 3 and 4).

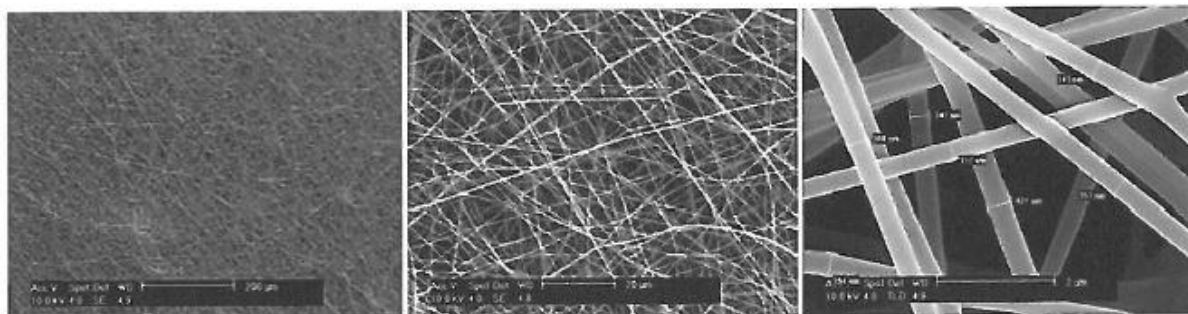


Fig. 3. Transformed nanofibers with a diameter in the range 300-400 nm.

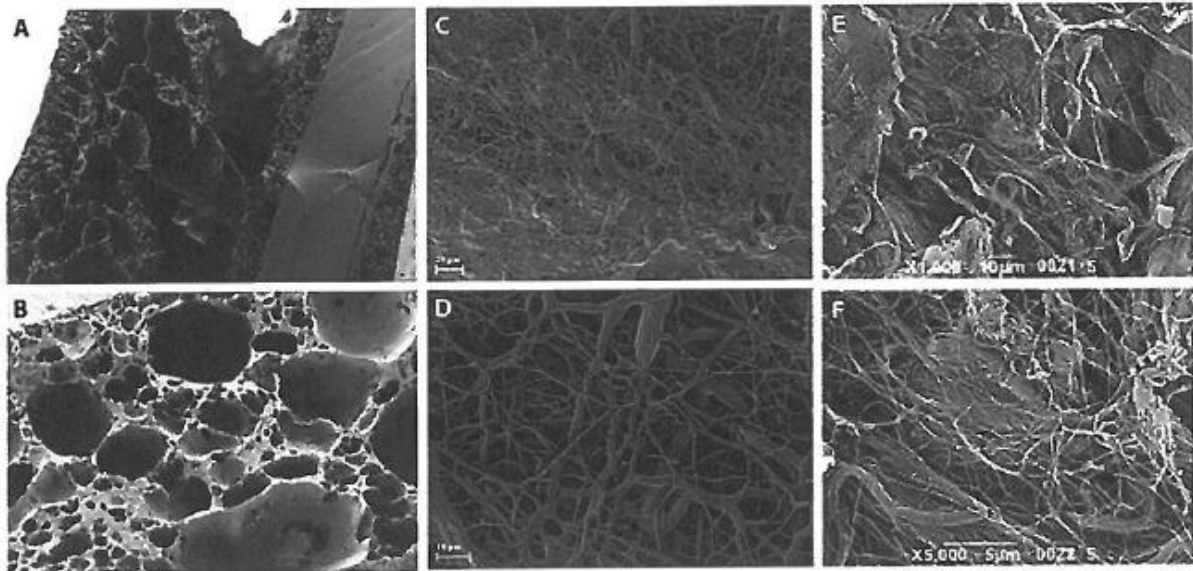


Fig. 4: Ultrastructural comparison of nanocomposite POSS - PCU (segments A, B) and PET (segments with C, D). PET nanocomposite better mimic the appearance of natural trachea after removal of the cells (segments E, F).

Recent published studies have shown that three-dimensional bioscaffold of PET is fully biocompatible with human hematopoietic cells and may even encourage the expansion of cells CD34+ ⁷. Additional data biocompatibility can be found in the Appendix at the end of the procedure description.

Extensive research in vitro and long-term in vivo PET research, in vivo on toxicity and biocompatibility have been conducted previously ^{7, 8, 9, 10, 11, 12, 13, 14}. Furthermore,

⁷ Feng Q, Chai C, Jiang X, et al. Expansion of engrafting human stem/progenitor cells hematopoietic in three dimensional scaffolds with surface-immobilized fibronectin. *J Biomed Mater Res A.*; 2006 September 15; 78 (4): 781-791.

⁸ Acocella F, Brizzola S, et al. Prefabricated tracheal prosthesis with partial biodegradable materials: a surgical and tissue engineering evaluation in vivo. *Journal of Science of Biomaterials; Polymer Edition*, 2007; 18 (5): 579-594.

⁹ Rainer and Centola M, et al. Comparative study of different techniques for the sterilization of poly-L-lactide electrospun microfibers: effectiveness vs. material degradation. *Intl Jour Artificial Organs* 2010; 33 (2): 76-85.

¹⁰ Kaschke, Gerhardt Bohm HJ, k, et al. Experimental in vitro and in vivo studies of epithelium formation on biomaterials seeded with several isolated cells. *J Invest Surg* 1996; 9:59 -79.

¹¹ Zhu, Leong MF, et al. Esophageal epithelium regeneration on fibronectin grafted poly (L-lactide-capralactone) (PIIC) nanofiber scaffold. *Biomaterials* 2007; 28 (5): 861-868.

¹² Komura M, Komura H. An animal model study for tissue-engineered trachea from pre-fabricated and biodegradable scaffold using chondrocytes to augment repair of tracheal stenosis. *J Ped Surg* 2008; 43 (12): 2141- 2146.

¹³ Tsukada, Matsuda S, et al. Comparison of bioabsorbable materials for use in artificial tracheal grafts. *Interactive Cardiovascular and Thoracic Surgery* 2009; 8 (2): 225-229.

biocompatible nanocomposite has been successfully used for transplantation, which was done in Sweden, at Karolinska University Hospital in November 2011. In the November study mononuclear cells were isolated from bone marrow aspirates and plated on bioscaffold via plasma sterilized bioreactor, which is described below. The results showed improved survival rate of cells (increased number of cells, improved orientation and the accumulation of extracellular matrix) in the PET bioscaffold compared to the POSS bioscaffold (Fig. 5 and 6).

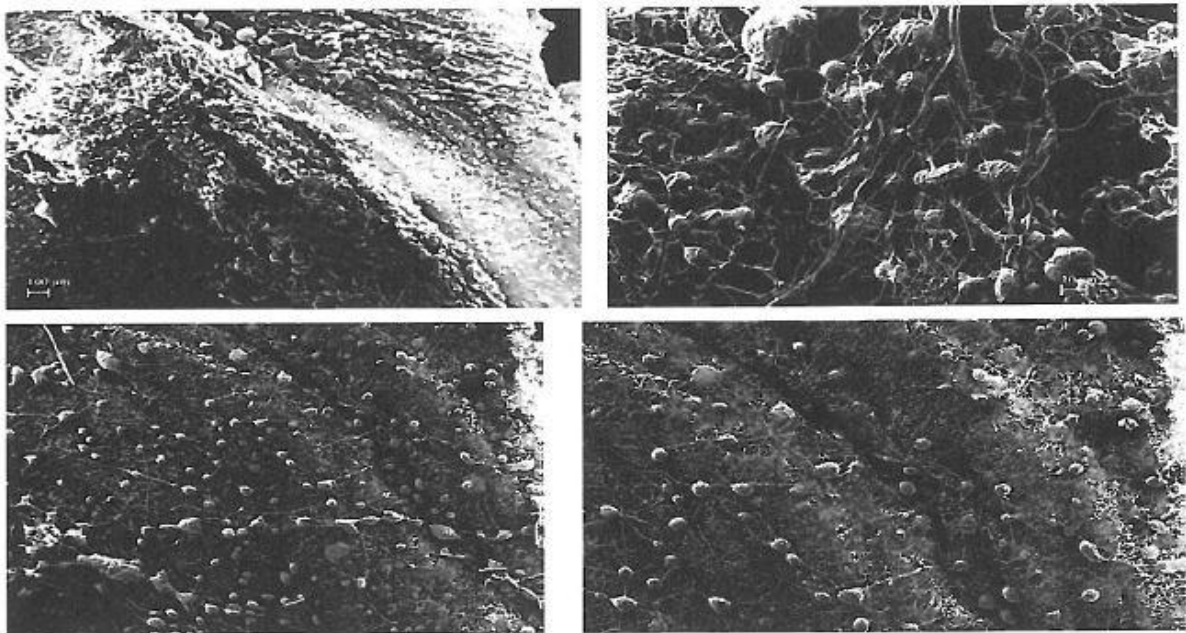


Fig.5: Structure of PET-nanocomposite after seeding with autologous progenitor cells

¹⁴ Kanzaki M, Yamato M, et al Tissue engineered epithelial cell sheets for the creation of a bioartificial trachea. *Tissue Engineering* 2006; 12 (5): 1275-1283.

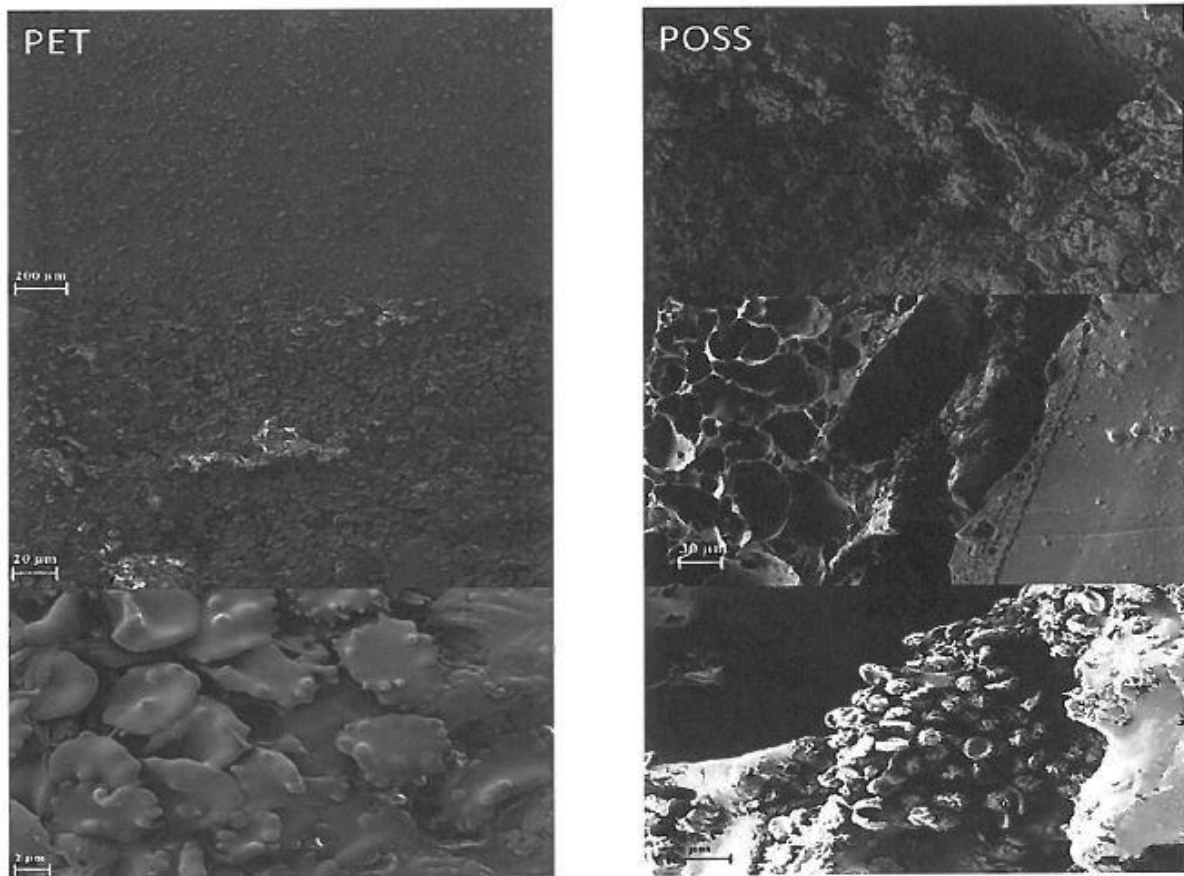


Fig. 6: Bioscaffold of PET gives a higher cell acceptability compared to bioscaffold of POSS - PCU.

We offer manufacturing of synthetic bioengineered trachea transplant for individual patients based on the results of recent computed tomography and endoscopic studies; the graft will be made of polymeric nanofibers transformed (Nanofiber Solutions®, Columbus, Ohio, United States), having mechanical and structural properties that mimic the natural respiratory tract (fig. 4). The company Nanofiber Solutions will manufacture the cartilage rings of the trachea with mechanical properties similar to those of a natural trachea with its resistance of mechanical collapse. The cartilaginous rings are sandwiched between the nanofibers and placed at regular intervals in a special form, exactly reproduced after the shape of the patient's trachea, and then transformed nanofibers will be used to cover each ring inside and outside, respectively.

The company Nanofiber Solutions does not see any problems in terms of manufacturing the device. As previously described, the inert nature of the polymer, combined with biomimetic topography transformed graft nanofibers provides the necessary surface properties for improved engraftment of cells, including mononuclear and epithelial cells specific to the trachea.

Bioreactor InBreath

This protocol includes the design of the bioreactor design previously used by our group during the first successful human implantation of a bioengineered trachea. The device, known by the market name InBreath 3D Organ Bioreactor (Harvard Bioscience, Holliston, Massachusetts), is placed inside the incubator for cell culture and consists of a single chamber of polysulfone, where the artificial organs are placed, as well as motor and remote control. Information about the materials used for the construction of the bioreactor can be found in Appendix 2.

The bioreactor InBreath chamber is easily separated from the motor block and can be subjected to plasma sterilization. The motor provides constant rotation of the cellular basis inside the chamber, thereby providing controlled effect of the hydrodynamic forces on the growing tracheal transplant. Protective chassis fully covers the brushless electric motor, protecting it from the corrosive action of moisture generated inside the incubator (fig. 7). Remote controller placed outside of the incubator, giving the possibility to adjust the speed rotation, without affecting the incubator.

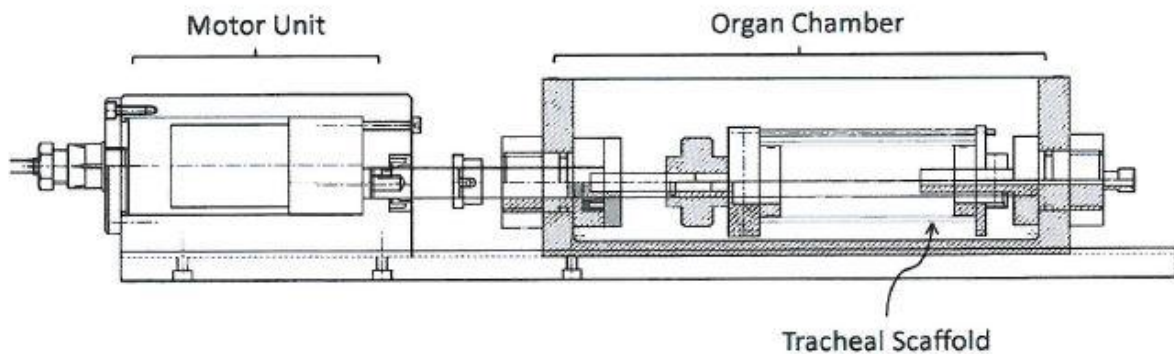


Figure 7: Bioreactor InBreath ® 3D Organ Bioreactor, Harvard Bioscience, Holliston, Massachusetts

According to this protocol, the synthetic bioscaffold will be specially manufactured for a patient using the synthetic material (PET). Both internal and external surfaces of the bioscaffold will be seeded with autologous undifferentiated mononuclear cells in the bioreactor.

Seeding cells on bioscaffold

1. Isolated MNCs are placed in a bag containing 300 ml DMEM (added with 10% human albumin) at temperature 4°C for transporting from the operation room to a laboratory working under the principles of GMP (Good Manufacturing Practice).
2. Bioscaffold (sterilized by ethanol or gamma radiation), bioreactor (plasma-sterilized) and surgical instruments (autoclaved) will be delivered in to a sterile room for cell culturing.
3. All persons who perform the manipulation of cells, bioreactor and bioscaffold must comply with Good Manufacturing Practices, including having sterile gloves, special protective clothing, etc.
4. The bioreactor is opened in a fume hood under sterile conditions and placed onto a sterile surface.
5. After that, the researcher must use a new pair of sterile gloves.

6. The bioscaffold will be removed from the primary sterile packaging and secured within the bioreactor at the respective fixtures.
7. After the bioscaffold has been docked within the bioreactor, MNCs (+ DMEM) will be seeded on the surface of bioscaffold.
8. 250 ml volume of medium (with the addition of autologous plasma and human albumin) will be added to the bioreactor chamber.
9. The following ingredients are added to the medium (see Appendix 3 "List of biological agents and characterization TGF β -3"): 10 mcg/ml of recombinant human transforming growth factor β -3 (R & D Systems, Minneapolis, Minnesota, United States), 10 nmol/l of recombinant parathyroid hormone-related peptide (PeproTech), 100 nmol/l dexamethasone, 10 μ g/ml of insulin (Sigma-Aldrich, Dorset, United Kingdom).
(Note: hereinafter products from other manufacturers may be used than the products from companies which have been used in previous transplants referred to).
10. Thereafter, the bioreactor will be placed in an incubator and the chamber closed (containing the bioscaffold, MNCs + 250 ml of medium).
11. NOTE: a bifurcate tracheobronchial bioscaffold does not perfectly fit to the shape of the chamber and this can lead to dynamic tension in the bioreactor. This tension (shear) will be transmitted through the external connection to the electric motor and can lead to termination of the chamber rotations. In that case, the bioreactor must manually be monitored every 20 minutes. In the case of a low speed rate due to influence of dynamic tension, it is possible to balance the chamber with an object to ensure continuous communication between these two components. In the event of a significant dynamic tension (shift), it is necessary to understand additional action to secure the bioscaffold. As a rule, such an event is adverse for tubular bioscaffolds.
12. The bioreactor is started with an initial speed of 1 rpm for 18 hours and then the rate will gradually be increased to 1.5 rpm.

13. After 24 hours, 50ml of the above medium is added into the chamber to a total volume of 300 ml.
14. After 48/72 hours, the chamber will be placed in a sterile container and gently moved to the operating room.

Transplantation of the airway

The patient will be subject to general anesthesia and intubated with an endotracheal tube. Any existing t-tube will be removed. The patient will also be subject to bone marrow aspiration, according to the procedure for preparing cells (see above). Median sternotomy will be carried out in supine position. After resection of the damaged airway segment the airway graft will be introduced (conditioning) with growth factors, including 10 ng/ml recombinant human transforming growth factor β -3 (R & D Systems, Minneapolis, Minnesota, United States), 10 nmol/l recombinant parathyroid hormone-related peptide (PeproTech), 100 nmol/l dexamethasone, 10 μ g/ml insulin (Sigma-Aldrich, Dorset, United Kingdom, G-CSF (of 1 μ g/kg) and erythropoietin (40000 IU), to stimulate the mobilization of peripheral hematopoietic cells.^{3,14} The graft will be adjusted in size, and then proximally and distally anastomosed to correct the defect of the respiratory tract by using non-absorbable sutures, such as Cardionyl 3/0 (Peters Surgical). The graft will then be covered by major omental flap wrapping (vascularized adipose tissue is separated from large stomach bend and the right gastro-omental artery and diaphragmatically or substernal transferred to the mediastinum) to provide long-term protection of the graft anastomoses, and indirect stimulate neovascularization of the graft.

Sterility tests

The risk of bacterial/fungal medium contamination will be assessed by microscopic examination prior to the transplantation. The quality of all components and medium are controlled by the manufacturers, and remain sealed until the cell seeding procedure.

Subsequent quality assessment of the medium is carried out by completion of the bioengineering process (Table 2).

Table 2 - Tests and acceptance criteria

Анализ	Критерий приемлемости / Метод
Стерильность	Стерильно / Критерии стерильности (Евр. Ф. 2.6.1)
Эндотоксины	<0,5 эндотоксиновых единиц/мл / Лал-тест
Микоплазма	Микоплазма не обнаружена / Культивация

*British Pharmacopoeia Volume IV. Appendix XVI a. Test for Sterility

A sample of the culture medium with the culture of MNCs will be added to the vial cultivation for growing blood cells shortly before the incubation procedure. The appearance of any foreign cultures in these samples within the next 48-72 hours will be deemed to be a significant event and may result in termination of the entire procedure. During the incubation period in the bioreactor, 24 hours after opening of the bioreactor, fresh medium is added to the growing blood cells and samples of fluid from the bioreactor are incubated to check for contamination. On the day of surgical implantation, as mentioned above, the neo-trachea is evaluated for cell growth and coating of the bioscaffold surface; at this point, samples from the medium from the bioreactor are sent for STAT analysis and Gram-staining, as well as being stored in vials for cultivating blood cells. If Gram staining gives a negative result, the graft will be considered as microbiologically sterile and ready for implantation into the patient's body. A small piece of the graft will be selected in the operating room prior to implantation for analysis of cellular culture. It will be placed in a tube for a standard smear and subjected to the standard analysis for [...] fluid from the wound. All samples will be analyzed a total of 14 days to evaluate the availability microbacteriological and fungal infections. Certificates of analysis will be included in the reports.

Postoperative procedures

To stimulate the process of regeneration in the postoperative period, the patient will receive pharmacological agents with following systemic injections:

- a) Recombinant analogues of G-CSF (Filgrastim, 10mcg/kg/day, no more than 30 mcg/kg/day)
- b) Synthetic analogues of erythropoietin (EPO alpha or beta, max40000 IU).

Both of these factors will be administered in adequate concentrations (in reduced doses not associated with any side effects) for stimulating mobilization and transformation of progenitor stem^{3,4,5,15,16} and bone marrow cells [...] day automatic controlled plasma erythropoietin level and calculation of whole blood (including leukocyte blood count). Levels above 50000-60000 cells in the blood will be considered a manifestation of toxicity and as a result will be reduced dose or discontinued. The treatment is carried out every other day for two weeks after transplantation..

Follow-up

Follow-up will include:

- Endoscopy (flexible or rigid bronchoscopy) of the transplanted respiratory system every day or every other day (when clinically indicated) during the first week and at least once before discharge from the hospital.

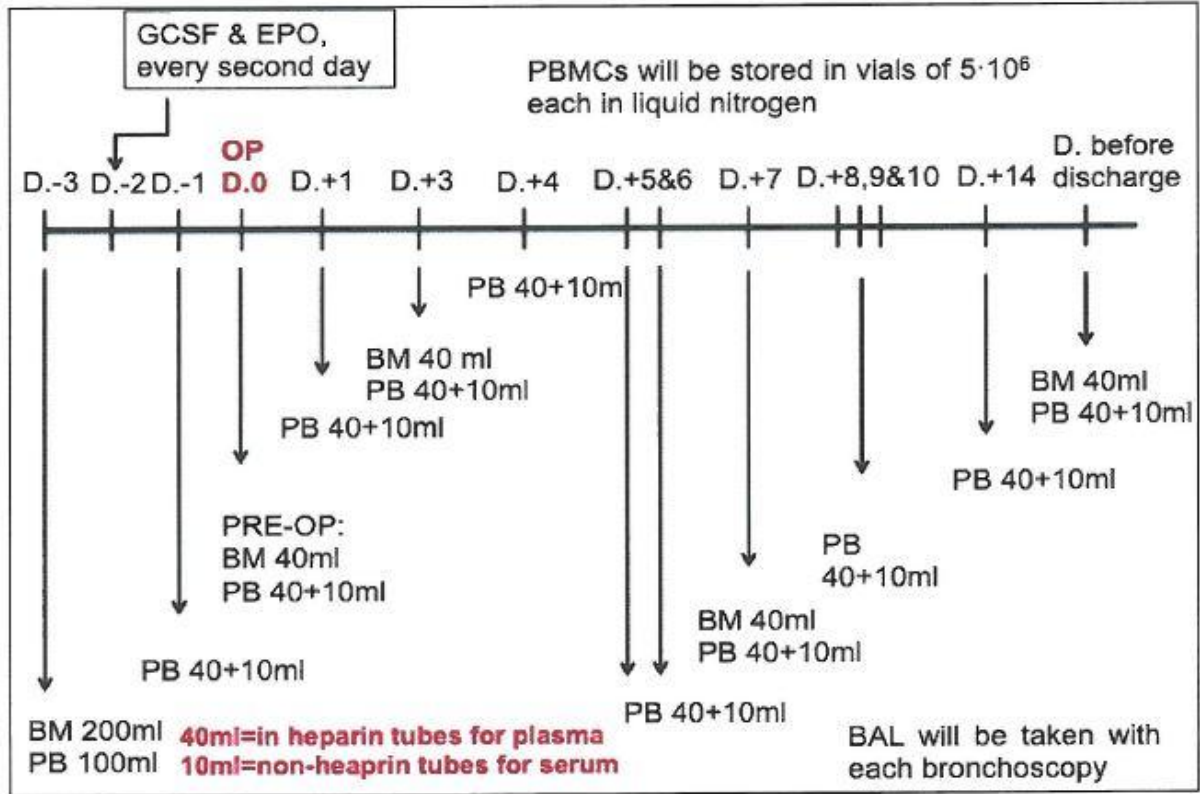
Bronchoscopy is performed on a monthly basis for the first six months and then every 6 months for the first 5 years. Samples from the respiratory airway mucosa shall be collected and stored for quality analysis.

- Counting of blood cells, (including leukocyte blood count) every day during the first two weeks.

¹⁵ Bader, A. Macchiarini P. Moving towards in situ tracheal regeneration: the bionic tissue engineered transplantation approach. J Cell Mol Med 2010; 14 (7): 1877-89.

¹⁶ Jungebluth P, Moll G, Baiguera S, Macchiarini P. Tissue engineered airway: a regenerative solution. Clin Pharm Ther 20 12; 91:81-93

- Evaluation and calculation of mobilized progenitor cells according to the graph below:



- Assessment of immunogenicity. After 3, 7 and 30 days after transplantation blood samples will be taken for analysis of HLA by OIA antibodies. Subsequent immunogenic studies will also be performed on 3, 6 and 12 months after transplantation.
- Computer tomography of the neck and chest with three-dimensional reconstruction of the transplanted airway will be done during the first, third, and sixth months during follow-up, and then every 6 months within the first 5 years.
- Subsequent cancer surveillance in children will be performed throughout the patient's life and include standard examinations.

9.0 Expected risks

The positive effect of this operation is supposed to be greater than the risk since this procedure may be the only possible chance of cure for some patients. Training will be held in appropriate cell laboratory in compliance with good manufacturing practices. In addition, numerous sterility tests will be carried out on all cells and materials before tracheal transplantation. If the sterility of cells and materials will be called into question, which seems unlikely, the whole procedure will immediately be terminated.

10.0 Adverse events

Possible complications of the transplant are postoperative bleeding, injury to the nervus recurrence, respiratory infections, anastomotic leak, wound infection, respiratory failure and the need for mechanical ventilation. All adverse events should be documented and communicated to the lead researcher, the sponsor and the Ethics Committees of the University and the Hospital. Address and phone number of the clinic for emergency GBUZ "Regional clinical hospital (N) 2 (I) S.V. Ochapovsky Department of Health Care Krasnodar Krai "at the address: 350086, Krasnodar, street. May Day, d. 167, Tel.:(861) 252-73-02, 260-35-11, Head of the Oncology Department, doctor of higher category, PhD Polyakov, Igor Stanislavovich

11.0 Introduction of amendments

The clinical trial plan cannot be changed without the written permission from the lead researcher, the funder, the Ethical Committee of the University and the Clinic. Amendments may require regulatory approval prior to their entry into force. All the amendments to the protocol, the patient's informed consent map, must be approval by the Ethical Committee of the University and the Clinic.

12.0 Publication policy

The results of this research can be used for publication.

13.0 Individual registration card (separate application)

Appendix 1: Biocompatibility, acceptability / cell proliferation and mechanical characteristics of PET medical devices available on the market at present time.

Bioscaffold material: polyethylene terephthalate (PET) is not absorbable.

Summary on biocompatibility: polyethylene terephthalate has for more than a decade successfully been used in fabrication of numerous of surgical implants and medical devices, from intracardiac and vascular grafts to threads for surgical sutures. The company Nanofiber Solutions (Columbus, Ohio) has developed tracheal and tracheobronchial bioscaffolds imitating the nanofiber structure of the natural trachea (Fig. 8). These bioscaffolds are made of non-absorbable polyethylene terephthalate transformed into nanofibers with an mean diameter of 350 nm.

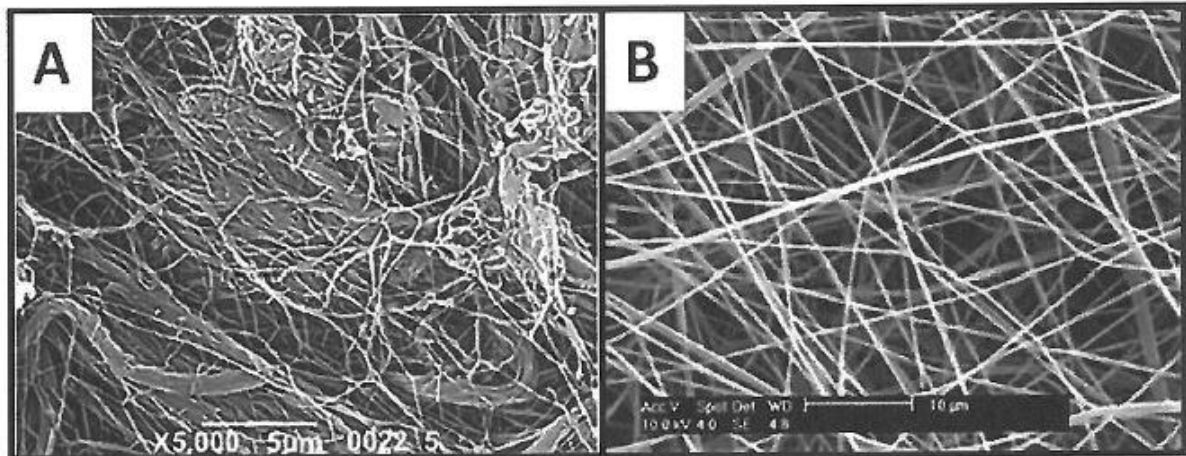


Fig. 8. Electron micrograph scanning of purified human tracheal cells (A) and artificial PET trachea manufactured by the company Nanofiber Solutions (B).

An artificial tracheobronchial graft using bioscaffold from PET-material has already been successfully transplanted at Karolinska University Hospital, Stockholm, Sweden, in November 2011. The clinical success of this operation indicates that the tracheobronchial graft of bioengineered nanocomposite and autologous mononuclear cells may be the only chance of cure for some patients.

We propose to use the same material for the bioscaffold and the same procedure for its production, which has been successfully used for the tracheal transplantation in November,

2011, using this Protocol. The polymer nanocomposite PET has carefully been studied on cell compatibility, and recent surgery, performed at Karolinska University Hospital, demonstrated its acceptability, ability to allow for proliferation of autologous mononuclear cells and early (7 days) re-epithelialization with respiratory epithelium.

Conclusion: information on the biocompatibility of implantable prostheses made of PET-material such as spinal cord, esophagus and cardiac valves is provided in the table below. All data confirm excellent biocompatibility when using the material for medical implants for vital organs. These data, and also the successful tracheal transplantation in a patient in November 2011, have shown excellent biocompatibility, allow concluding that the proposed bioscaffold material meets the requirements of biocompatibility and is safe for use.

Data on biocompatibility of medical devices made from PET, at the present available on the market.

Тест на биосовместимость Per ISO 10993	Критерии приемлемости	Ссылка #1—спинальный имплантат Zimmer Dynesys (FDA 510(k) K092234)-Компонент спинного мозга ¹⁷	Ссылка #2—эндоскопический имплантат Enteryx™ для лечения гастроэзофагеальной рефлюксной болезни (FDA PMA P020006) ¹⁸	Ссылка #3—сердечный чрезкатетерный клапан Edwards Sapien™ (FDA PMA P010041) ¹⁹	Ссылка #4—Carpentier-Edwards™ S.A.V.™ биопротез, модель 2650 (аортный) (FDA PMA P10041) ²⁰
Цитотоксичность	0, 1 или 2 (нетоксично)	СООТВЕТСТВУЕТ (0 для всех)	СООТВЕТСТВУЕТ (Отсутствие признаков токсичности)	СООТВЕТСТВУЕТ (нетоксично)	СООТВЕТСТВУЕТ

¹⁷ Zimmer Spine FDA Executive Summary, 2009, for K092234: <http://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/medicaldevices/medicaldevicesadvisorycommittee/orthopaedicandrehabilitationdevicespanel/ucm188734.pdf>

¹⁸ Medical Technologies Enteric FDA Summary of Safety and Effectiveness, 2003, for P020006: <http://www.fda.gov/ohrms/dockets/ac/03/briefing/3921/SSSED.pdf>

¹⁹ Edwards SAPIEN Transcatheter Heart Valve™ FDA Summary of Safety and Effectiveness, 2011, for P010041: <http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/CirculatorySystemDevicesPanel/UCM262936.pdf>

²⁰ Carpentier-Edwards S.A.V.™™ Bioprosthesis, Model 2650 (Aortic) FDA Summary of Safety and Effectiveness, 2002, P010041: http://www.accessdata.fda.gov/cdrh_docs/pdf/P010041b.pdf

Тест на биосовместимость Per ISO 10993	Критерии приемлемости	Ссылка #1— спинальный имплантат Zimmer Dynesys (FDA 510(k) K092234)- Компонент спинного мозга ¹⁷	Ссылка #2— эндоскопический имплантат Enteryx™ для лечения гастроэзофагеальной рефлюксной болезни (FDA PMA P020006) ¹⁸	Ссылка #3— сердечный чрезкатетерный клапан Edwards Sapien™ (FDA PMA P010041) ¹⁹	Ссылка #4— Carpentier-Edwards™ S.A.V.™ биопротез, модель 2650 (аортный) (FDA PMA P10041) ²⁰
Острая системная токсичность	Тестируемый имплантат должен продемонстрировать ≤ биологическую реакцию по сравнению с контрольной группой (мыши); < 2 мышей могут иметь признаки токсичности; < 3 мышей могут продемонстрировать потерю веса >2 г	образов; нетоксичный) СООТВЕТСТВУЕТ (не наблюдаются признаки токсичности и потери веса >2 г)	СООТВЕТСТВУЕТ (удовлетворяет критериям Фарм. США (USP))	СООТВЕТСТВУЕТ (Пикапы изменений в течение 72 часов)	(ингибирование - 0%) СООТВЕТСТВУЕТ (патологично)
Тест на раздражение	Показатель основного первичного раздражителя (PII) Шкала от 0 – 0,4 (незначительный); не вызывает раздражения	СООТВЕТСТВУЕТ (не вызывает раздражения; PII: категория раздражения –0)	СООТВЕТСТВУЕТ (удовлетворяет критериям USP)	СООТВЕТСТВУЕТ (не вызывает раздражения)	СООТВЕТСТВУЕТ (не вызывает раздражения)
Тест на мутагенность	Менее, чем двукратное увеличение числа ревертантных колоний на чашку по сравнению со средним числом колоний контрольной группы для каждого штамма	СООТВЕТСТВУЕТ (немутагенный; менее, чем двукратное увеличение для каждого штамма)	СООТВЕТСТВУЕТ (негативные результаты)	СООТВЕТСТВУЕТ (негативные результаты)	Не проводится

Тест на биосовместимость Per ISO 10993	Критерии приемлемости	Ссылка #1—спинальный имплантат Zimmer Dynesys (FDA 510(k) K092234)-Компонент спинного мозга ¹⁷	Ссылка #2—эндоскопический имплантат Enteryx™ для лечения гастроэзофагеальной рефлюксной болезни (FDA PMA P020006) ¹⁸	Ссылка #3—сердечный чрезкатетерный клапан Edwards Sapien™ (FDA PMA P010041) ¹⁹	Ссылка #4—Carpendier-Edwards™ S.A.V.™ биопротез, модель 2650 (аортный) (FDA PMA P10041) ²⁰
Аллергическая проба	Класс <1 или отсутствие кожной аллергической реакции, превышающей реакцию контрольной группы. С использованием модели Magnusson-Klingman, Класс I, Класс аллергической реакции «Слабый аллерген»	СООТВЕТСТВУЕТ (реакция не превышает 0; аллергическая реакция 0%); классифицируется как Класс I (Слабый аллерген)	СООТВЕТСТВУЕТ (Класс I Слабая реакция, эквивалентна негативному контролю)	СООТВЕТСТВУЕТ (не вызывает аллергической реакции)	СООТВЕТСТВУЕТ (не вызывает аллергической реакции)
Тест на пирогенность	Температурная разница не превышает 0,5 °C от исходной температуры через 1-3 часа после введения	СООТВЕТСТВУЕТ (непирогенный; у животных не наблюдалось повышения температуры более чем на 5 градусов Цельсия)	Не проводился	СООТВЕТСТВУЕТ (не было зарегистрировано повышения или изменения температуры)	Не проводился
Тест на генотоксичность/мутации клеток млекопитающих	Немутagenный, если изолированная культура тестируемого образца имеет частоту мутаций менее, чем двукратное увеличение для раствора контрольной группы	СООТВЕТСТВУЕТ (экстракты не проявили мутагенности)	СООТВЕТСТВУЕТ (негативные результаты)	СООТВЕТСТВУЕТ	Не проводился
Анализ aberrаций хромосом	Немутagenный, если значение $p > 0,05$ для тестируемого	СООТВЕТСТВУЕТ (немутагенный; нет)	СООТВЕТСТВУЕТ (негативные результаты)	СООТВЕТСТВУЕТ (немутагенный)	СООТВЕТСТВУЕТ (немутагенный)

Тест и биосовместимость Per ISO 10993	Критерии приемлемости	Ссылка #1—спинальный имплантат Zimmer Dynesys (FDA 510(k) K092234)-Компонент спинного мозга ¹⁷	Ссылка #2—эндоскопический имплантат Enteryx™ для лечения гастроэзофагеальной рефлюксной болезни (FDA PMA P020006) ¹⁸	Ссылка #3—сердечный протезотерный клапан Edwards Sapien™ (FDA PMA P010041) ¹⁹	Ссылка #4—Carpenfier-Edward™ S.A.V.™ биопротез, модель 2650 (аортный) (FDA PMA P10041) ²⁰
	образца по сравнению с контрольной группой с негативным уровнем аберрации	статистически значимого увеличения в уровне аберрации)			
Подкожная имплантация	Разница между средним показателем тестируемого образца и средним показателем контрольной группы равняется степени инкапсуляции; не вызывает раздражения, если разница равняется нулю	СООТВЕТСТВУЕТ (не вызывает раздражения; разница гистопатологических показателей равна 0)	СООТВЕТСТВУЕТ (удовлетворяет критериям USP)	СООТВЕТСТВУЕТ (не вызывает раздражения)	Не проводился
Подострая токсичность	2х-недельное исследование токсичности в внутривенном введении препарата мышам; нетоксичный	Не проводился	СООТВЕТСТВУЕТ (нетоксичный)	СООТВЕТСТВУЕТ (нетоксичный)	Не проводился
Хроническая токсичность	Оценка внутримышечного имплантата у кролика; нетоксичный	Не проводился	СООТВЕТСТВУЕТ (нетоксичный; слабая воспалительная реакция через 1 год)	СООТВЕТСТВУЕТ (нетоксичный после 90 дней)	СООТВЕТСТВУЕТ (нетоксичный после 90 дней)
Канцерогенность	transgenic модель мыши — 6 месяцев; неканцерогенный	Не проводился	СООТВЕТСТВУЕТ (неканцерогенный)	Не проводился	Не проводился (так как тестируемые образцы не прошли мутагенного потенциала)
Гемосовместимость	Не	Не проводился	Не проводился	СООТВЕТСТВ	СООТВЕТСТВ

Тест на биосовместимость Per ISO 10993	Критерии приемлемости	Ссылка #1—линейный имплантат Zimmer Dynesys (FDA 510(k) K092234)-Компонент спинного мозга ¹⁷	Ссылка #2—эндоскопический имплантат Enteryx™ для лечения гастроэзофагеальной рефлюксной болезни (FDA PMA P020006) ¹⁸	Ссылка #3—сердечный чрезкатетерный клапан Edwards Sapien™ (FDA PMA P010041) ¹⁹	Ссылка #4—Carpenier-Edwards™ S.A.V.™ биопротез, модель 2650 (аортный) (FDA PMA P10041) ²⁰
ть	наблюдалось гемолиза <i>in vitro</i> ; время свертывания не изменилось			УЕТ (не повлиял на гемолиз и время свертывания)	ВУЕТ (не повлиял на гемолиз и время свертывания)
Выводы	УДОВЛЕТВОРИТЕЛЬНО	СООТВЕТСТВУЕТ: Степень поражения тканей в течение испытательного периода не изменилась. ПЭТ-частицы не вызвали никаких иммунологических реакций, включая индукцию остеолитиса; системной токсичности не наблюдалось. Скорость и качество заживления тестируемых и контрольных животных были сходными и соответствовали на каждом этапе исследования.	СООТВЕТСТВУЕТ: Долгосрочное (12 месяцев) исследование эндоскопического имплантата Enteryx на собаках продемонстрировало, что ПЭТ-материал может быть безопасно введен собакам с минимальными реакциями при длительном использовании.	СООТВЕТСТВУЕТ: The Сердечный чрезкатетерный клапан SAPIEN модели 9000TFX удовлетворяет всем требованиям биосовместимости.	СООТВЕТСТВУЕТ: Клапан модели 2650, материал и пленка из ПЭТ, были успешно протестированы на биосовместимость. Результаты <i>in vivo</i> имплантации на животных и долгосрочных клинических испытаний S A V клапана на безопасность подтверждают биосовместимость готового устройства

Engraftment and cell proliferation

PET polymer has been extensively analyzed for cellular compatibility and proved its ability to effectively support the implantation of cells, their dispersion and attachment for exogenous agents and preservation of the phenotype of cells, and was shown to have biomechanical dynamic ability to handle stress.²¹ In addition, bioscaffold, consisting of

transformed nanofibers have demonstrated the ability to provide relevant micro-habitation for [...] and [...] differentiation of progenitor cells.²²

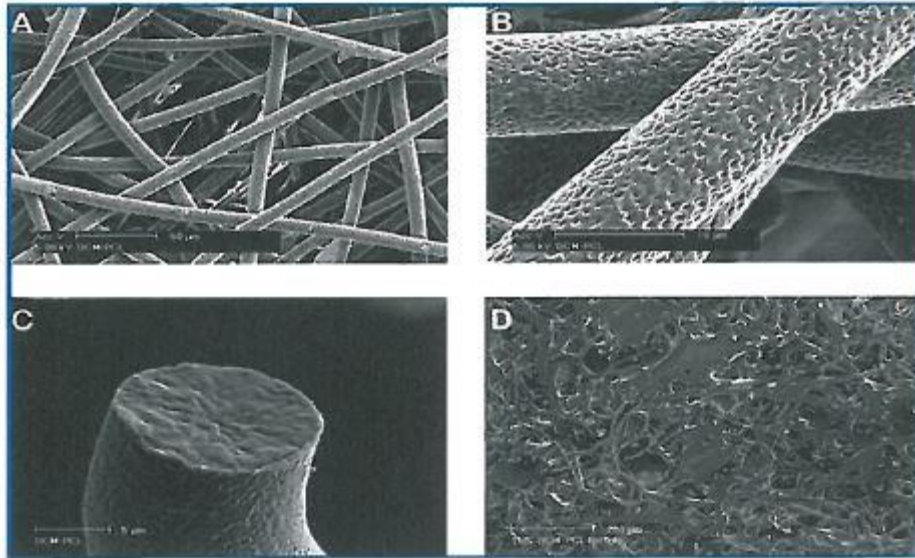


Fig. 9. PET bioscaffold of transformed nanofibers shows: (A) original microstructure fiber network (B) nano-pores on the surface of the fibers that contribute to cell attachment, (C) section fiber-demonstrates a lack of pores within it, (D) relatively dense flat bottom surface bioscaffold for slower cell migration cell in the spot with medium.

²¹ Nam J, Rath in Knobloch, TJ, Lannutti JJ, Agarwal S. Novel electrospun scaffolds for the molecular analysis of chondrocytes under dynamic compression. *Tissue Eng Part a*. 2009; 15 (3): 513-23.

²² Nam J, Johnson J, Lannutti JJ, Agarwal S. Modulation of embryonic progenitor cell differentiation mesenchymal via control over pure mechanical modulus in electrospun nanofibers. *Acta Biomater*. 2011; 7 (4): 1516-24.

Mechanical characteristics of the bioscaffold

SPECIMEN Electrospun bifurcated * scaffold produced by Nanofiber Solutions, Columbus, OH.

TEST Uniaxial tensile test.

CONDITIONS Universal testing machine Lloyd LRX
Load cell: 2500 N
Preload: 1 N
Speed of testing: 1 mm/s
Stainless steel custom-made grips fixed to the rigid rings of the scaffold.
In order to prevent slippage, grips were pre-glued to the sample.
Tests were carried out on both as-received scaffolds and scaffolds sterilized by immersion in ethanol overnight and then dried for 24 h.
For each specimen 5 measurements were performed.

Measured parameters: Force at break (Fmax).
Elongation at break, defined as the percentage increase in length, before the break occurs, with respect to the initial length of the specimen.

ИЗДЕЛИЕ

Раздвоенный биокаркас из трансформированных нановолокон, произведенный компанией Nanofiber Solutions, г. Колумбус, Огайо, США

АНАЛИЗ

Испытание на одноосное растяжение

УСЛОВИЯ

Универсальная испытательная машина Lloyd LRX

Нагрузка ячейке: 2500 Н

Предварительная нагрузка: 1 Н

Скорость растяжения: 1 мм/с

Специальные зажимы из нержавеющей стали прикрепляются к твердым кольцам биокаркаса. Для предотвращения соскальзывания зажимы приклеиваются к биокаркасу.

Анализ был проведен на обоих полученных биокаркасах, биокаркасы были стерилизованы

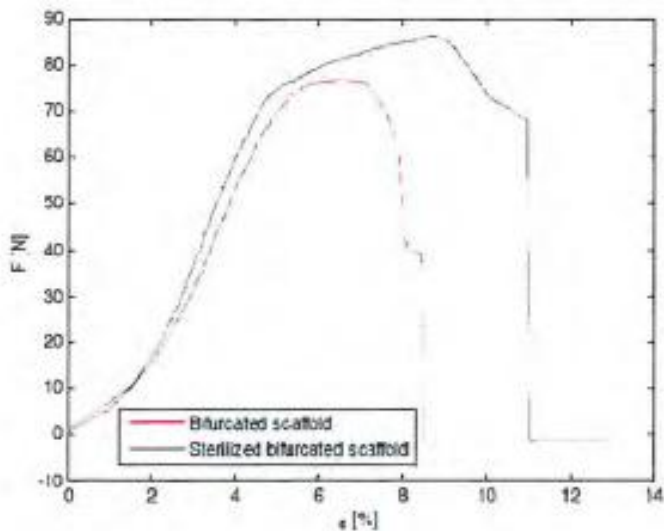
путем погружения в этанол на одну ночь, а затем высушены в течение 24 часов. Для каждого изделия было выполнено по 5 измерений.

Измеренные параметры: Сила разрыва (F_{max})

Растяжение при разрыве, определяемое как процент увеличения длины перед моментом разрыва, по отношению к исходной длине изделия.

Ниже: Раздвоенный биокаркас из трансформированных нановолокон





Вверху слева: Типичные графики зависимости растяжения от силы и графики силы при разрыве для тестируемого биокаркаса до и после гамма-стерилизации.

Анализ гамма-стерилизованного биокаркаса показал отсутствие каких-либо изменений размера, цвета, прочности на разрыв, или структуры материала согласно сканирующей электронной микроскопии, как показано ниже:

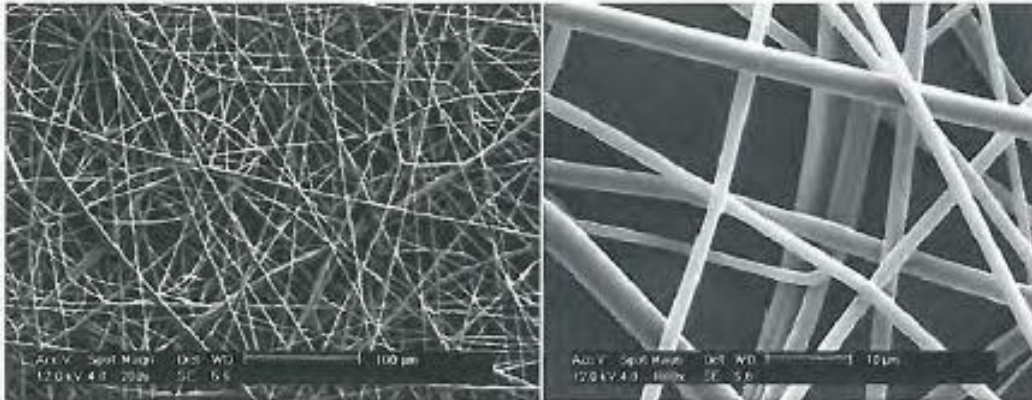
До стерилизации



После стерилизации



Размеры биокаркаса	Исходные	После гамма-стерилизации
Длина	6,1 см	6,1 см
Ширина	2,9 см	2,9 см



Вверху: СЭМ фотографии нановолоконного материала до стерилизации гамма-облучением.
Внизу: СЭМ фотография нановолокон после стерилизации гамма-облучением (28kGy).



Данные измерений предела прочности при растяжении (n=5):

	Средняя (МПа)	Стандартное отклонение	Стандартная погрешность
Контрольный образец	2,01	0,34	0,15
Образец после стерилизации гамма-облучением (28 kGy)	1,77	0,39	0,17

Приложение 2: Материалы биореактора (различные виды медицинских пластмасс и нержавеющая сталь)

Резюме: Медицинские материалы успешно применяются в целом ряде медицинских устройств, включая имплантаты, уже более двадцати лет. Используемый в данном клиническом исследовании биореактор помогает осуществить поддержку биокаркаса и обеспечить процесс выращивания клеток на его поверхности перед имплантацией. Биореактор изготовлен в основном из нескольких видов медицинских пластмасс, удовлетворяющих критериям Фарм. США (USP) Класса VI биосовместимости (Класс б) (см. таблицы ниже). Основные материалы, покрывающие поверхность биореактора, полиоксиметилен (POM-C) и полисульфон (PSU), уже использовались ранее для культивирования двух искусственных трансплантатов трахеобронхиального дыхательного пути, операции по пересадке которых были успешно проведены в Госпитале Каролинского университета, Стокгольм, Швеция, в июне и ноябре 2011 года.

Заключение: Применение материалов Класса VI USP (медицинского класса/Класса б) в сочетании с успешным клиническим исходом вышеупомянутых операций в июне и ноябре 2011 года указывает на то, что биореактор удовлетворяет критериям биосовместимости как аппарат для предоперационной обработки и процедуры переноса клеток на биокаркас в соответствии с протоколом для трахейного трансплантата.

Материалы, использованные в конструкции биореактора:

Компонент	Химический материал	Торговое название	Производитель	Удовлетворяет USP, Класс б (Медицинский материал)
Уплотнения, статические	EPDM резина	70 EPDM 291	Freudenberg Process Seals GmbH & Co. KG, Германия	Да
Уплотнения, динамические	Фторнаучук (FKM)	FKM 80.445-01	Angst+Pfister AG, Швейцария	-

Компонент	Химический материал	Торговое название	Производитель	Удовлетворяет USP, Класс 6 (Медицинский материал)
Зажимы	X10 CrNi 18-8 (нержавеющая сталь)	1.4310	AGILFedern, Германия	Нержавеющая сталь
Контейнер	Полисульфон (PSU)	TECASON™ S	Ensinger Inc, США	Да
Крышка для контейнера с культурой	Полисульфон (PSU)	TECASON™ S	Ensinger Inc, США	Да
Переносная крышка	Полисульфон (PSU)	TECASON™ S	Ensinger Inc, США	Да
Переносной контейнер	Полиоксиметилен сополимер (POM-C)	Centrodal C	Centroplast Engineering Plastics GmbH, Германия	Да
Крышка переносного контейнера	Полипропилен (PP)	CentrolabHT™	Centroplast Engineering Plastics GmbH, Германия	Да
Вращающиеся детали	Полиоксиметилен сополимер (POM-C), Полиэфирэфиркетон (PEEK)	Centrodal C, PEEK LSG	Centroplast Engineering Plastics GmbH, Germany Schmidt + Bartl GmbH	Да
Вал привода	X2 CrNiMo 18-15-3 (нержавеющая сталь)	1.4441 ESU	EZMEdelstahlZieherei Mark GmbH, Германия	Не применимо
Оправка	X6 CrNiMoTi 17-12-2 (нержавеющая сталь)	1.4571	BSGStahlhandel, Германия	Не применимо

Результаты тестов по биосовместимости для материалов PEEK и CentrolabHT™, проведенных производителями (выполнение критериев Класса 6 USP подразумевает тестирование материалов на острую системную токсичность, внутрикожную реакцию и приживление):

Тест на биосовместимость Согласно ISO 10993	Критерии приемлемости	Материал PEEK	Материал CentrolabHT™
Острая системная токсичность	Тестируемая группа должна продемонстрировать ≤ биологическую реакцию по сравнению с контрольной группой (мыши); < 2 мышей могут	УДОВЛЕТВОРЯЕТ (Отсутствие признаков токсичности и потери веса >2 г)	УДОВЛЕТВОРЯЕТ (удовлетворяет требованиям USP)

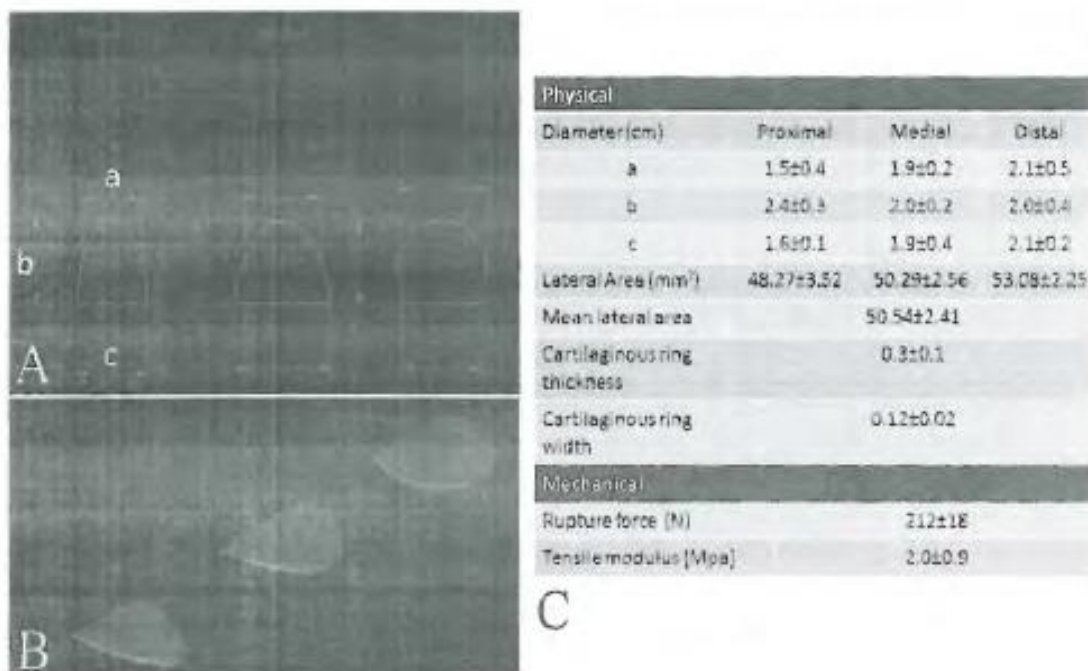
Тест на биосовместимость Согласно ISO 10993	Критерии приемлемости	Материал PEEK	Материал CentrolabHT™
	иметь признаки токсичности; < 3 мышей теряют вес >2 г		
Внутрикожная реакция через 72 часа	Должны удовлетворять требованиям минимальной реактивности	УДОВЛЕТВОРЯЕТ (минимальная реактивность)	УДОВЛЕТВОРЯЕТ (минимальная реактивность)
Тест на приживление (7 дней)	Нет реакции	УДОВЛЕТВОРЯЕТ (нет реакции)	УДОВЛЕТВОРЯЕТ (нет реакции)

Appendix 3: Characteristics of biological agents and factors TGF-β3

The following table presents the growth factors used preoperatively in the present protocol to accelerate tissue regeneration:

Factor	Activity	Company	Distributor in Sweden	Quantity	Intra-operative dose	Pre- and post-operative dose
Recombinant human factor TGF-β3, CF*	TGF-β3	R&D Systems	R&D Systems Europe Ltd. info@RnDSystems.co.uk www.RnDSystems.co.uk	10 µg	1 ampoule (5 µg)	1 ampoule (5 µg) preop.
Granulocyte colony-stimulating factor (G-CSF)	Filgrastim (Neupogen®)	AMGEN	Amgen Inc. One Amgen Center Drive Thousand Oaks California 91320-1799 USA	30 IU (0,6mg/ml)	1 ampoule (30 IU)	9 ampoules (30 IU)
NeoRecormon	Erythropoietin and recombinant Epoetin α	Janssen Biotech	Jansen Biotech, Inc. 800 Ridgeview Road Horsham, PA 19044	10000 IU or 40000 IU	4ampoules á 10000 IU (in total 40000 IU)	9 ampoules á 40000 IU
Recombinant human factor PTHrP	rhPTH	PeproTech	PeproTech SE Klarabergsviadukten 70, 107 24 Stockholm, Sweden	50µg	0,5 ampoule preop.	0,5 ampoule preop.
Insulin		Sigma Aldrich	Sigma-Aldrich Technical Services PO Box 14508 St. Louis, MO 63178 USA	10ug/ml	5 µg/ml	5 µg/ml
Dexamethasone		Sigma Aldrich	Sigma-Aldrich Technical Services PO Box 14508 St. Louis, MO 63178 USA	100 nmol/L	50nmol/L	50nmol/L

* TGF-β3: calculation of the necessary dose of TGF-β3 based on the size of the human trachea and as a rule, it is based on the average lateral area of the cartilage as shown below in figure 1B.



Используя вышеприведенный пример определения средней латеральной площади, было подсчитано, что требуемая концентрация TGF-β3 составит 0,1 мкг/мм² (примерно 5 мкг на кольцо). Это дает в итоге, что необходимо приготовить раствор 10 мкг/мл TGF-β3 и вводить по 0,5 мл на каждое хрящевое кольцо.

Ведущий ученый  П. Маккиарини