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Research Article

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Vascularized biocompatible synthetic tracheal matrix based on ultrafibrous polymeric material

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ABSTRACT

We aimed to prepare a biocompatible synthetic vascularized tracheal scaffold for substitution of natural tracheae with life-threatening lesions Six beagle-dogs were implanted with polymeric tracheal matrices, the grafting was heterotopic to the formed intramuscular pocket in the groin. The implants were colonized by recipients' multipotent mesenchymal stromal cells (MMSC) before grafting. Tracheal matrices was electrospun from nonwoven fabric by a unique experimental monocapillary device for electrospun. Tracheal scaffolds were extracted from the groins in a month after the implantation. The scaffolds were covered by connective tissue capsule intimately interconnected with their matrix and were highly vascularized. Implants based on the nonwoven tracheal scaffolds colonized with recipient`s MSC seem to be one of the most promising ways for trachea prosthesis.

Key words: Trachea, Matrix, Scaffold, *Mesenchymal multipotent* stromal cells, Dogs.

INTRODUCION

Replacement of serious post-operative tracheal defects still remains unsolved problem despite attempts to use for this purpose different combinations of autologous and allogeneic implants and synthetic materials as well [1-6]. P. Macchiarini [2] developed the method of matrix decellularization and repopulation with recipient's mesenchymal and epithelial cells. But according to author's method it takes 6 weeks to prepare an implant. That limits applying of this technology. It will be unusable for the patients with decompensated stenosis of trachea, cancer patients, and patients who need immediate surgical intervention. Moreover, the process of decellularization can be the cause of structural changes of trachea and thus worsen its biomechanical features [7]. There is a difficulty in selection of implants which satisfy the requirements of size and quality of donor`s material, the prevention of contamination the material with different microorganisms can be problematic too. Alternative way of getting tracheal implant is the method suggested by P. Delaere [8, 9]. This method includes additional step of donor's trachea prefabrication under subcutaneous implantation during 9 months. This way is too labour- and time-consuming, and requires immunosuppressive therapy. This has turned back to the current attempts of creating synthetic tracheal prosthesis with improved biocompatibility and cell conductivity. Promising trend is creating polymeric biocompatible matrixes colonized with recipient's precursor cells for reconstitution of trachea. In the last years there are tries to use for

clinical purposes synthetic tracheal scaffolds based on porous and fibrous polymers, colonized with recipient's cells. They have clear advantages over allogeneic transplants of trachea and allow to create individualized bioimplants for reconstitution of trachea defects [3, 5, 6]. However, because of long duration of the vascularization process infectious complications take place and can cause transplant rejection. One of the promising method is prefabrication of polymeric tracheal matrix with heterotrophic transplantation in order to vascularize and colonize it with recipient`s cells.

MATERIALS AND METHODS

Polymeric tracheal matrices for preclinical trials on dogs were obtained from nonwoven material by electrospun on the original experimental monocapillary setup for electrospun. As the polymer for nonwoven material was used fluoroplastic ("ChimCombinat", Russia). Semirings for reinforcing the nonwoven polymeric material were obtained by termopressing as the raw material was used polyurethane "Elastollan 1195A" (Elastogran, Germany) (Fig. 1).

Figure 1: Sample of tracheal matrix based on polymeric ultrafibrous materials for experiments on dogs

Six healthy dogs ranging from 15 kg to 22 kg in weight were studied. All animals received care in compliance with the "Guide for the care and use of laboratory animals" prepared by the Institute of laboratory animal resources, National research Council, and published by the National Academy Press, revised 1996. Researches have been made on beagle dogs after permission of Local Ethical Committee of NN Blokhin Russian Cancer Research Center. General anesthesia was induced with thiopental and maintained with oxygen and 1–3% halothane through an endotracheal tube. All animals were perfused with a crystalloid solution. Pulse and arterial pressure were monitored intraoperatively. Trachea matrixes were colonized with recipient`s cells for enhancement of biocompatibility and acceleration of integration. MMSC were generated from the cells gotten from bone marrow obtained by sternal puncture. The cells for bone marrow were placed in sterile culture flasks with culture medium RPMI 1640 (PanEco, Russia) containing 10 % fetal f serum (PanEco, Russia). Analysis of MMSC culture was performed using Axiovert 40 (Zeiss, Germany) in a transmission or phase contrast mode. Histological examination was performed by standard methods. Matrixes were implanted in the femoral intramuscular space folds. The operation was performed under general anesthesia with the requirements of aseptic and antiseptic – in terms of the operational department of Experimental Therapeutics Unit of NN Blokhin Russian Cancer Research Center. Watching the animals lasted for 1 month. During this period, daily visual inspection of the operation area, regular analysis of hematological parameters using the analyzer (ProCyte Dx, Netherlands) was carried out.

RESULTS AND DISCUSSION

Before implantation of the sterile matrices to the animals they were colonized with MMSC (Fig. 2) ex vivo obtained from the cells of recipient's bone marrow by co-cultivation in culture medium.

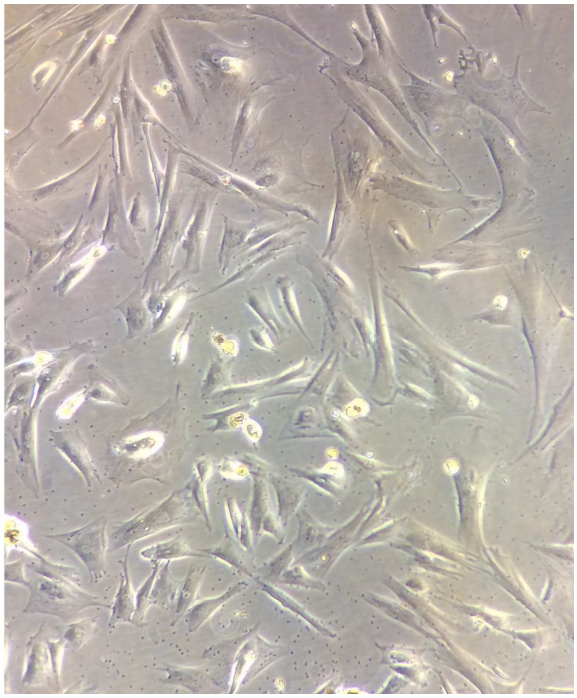


Fig 2a

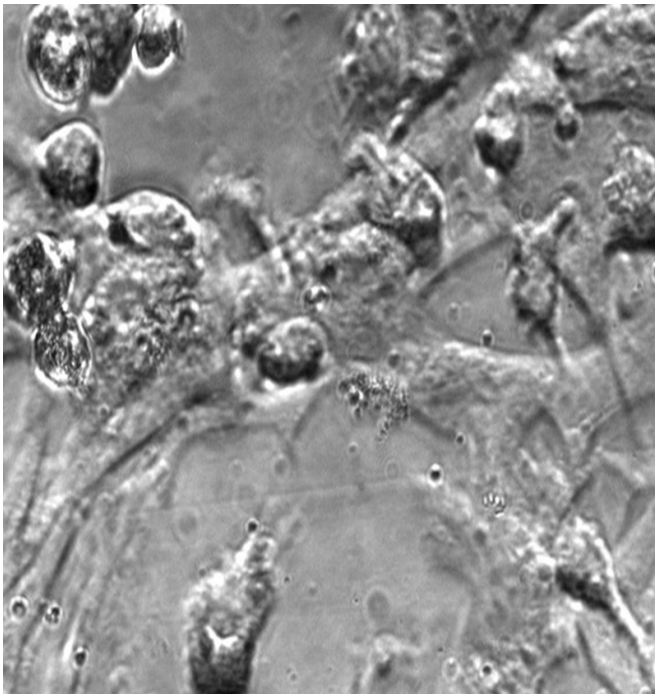


Fig 2b

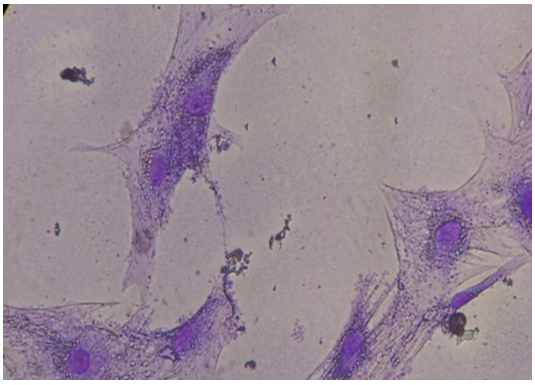


Fig 2c

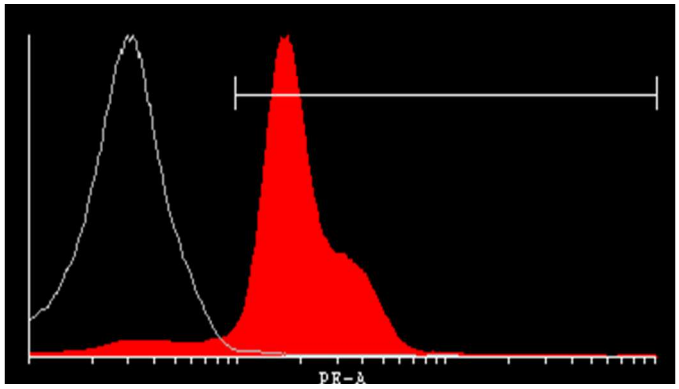


Fig 2d

Figure 2: **Dog's MMSC in culture, before tracheal matrix colonization**

Tracheal matrixes colonized with MMSC were heterotopic implanted to the dogs in the formed intramuscular pocket in the groin. There was formed an intramuscular pocket in the dogs where to sample of matrix was placed. After that the wound edges were stitched layers with the imposition of intermittent surgical sutures and external finishing of the seam and the surrounding area of skin antiseptics (Fig. 3).

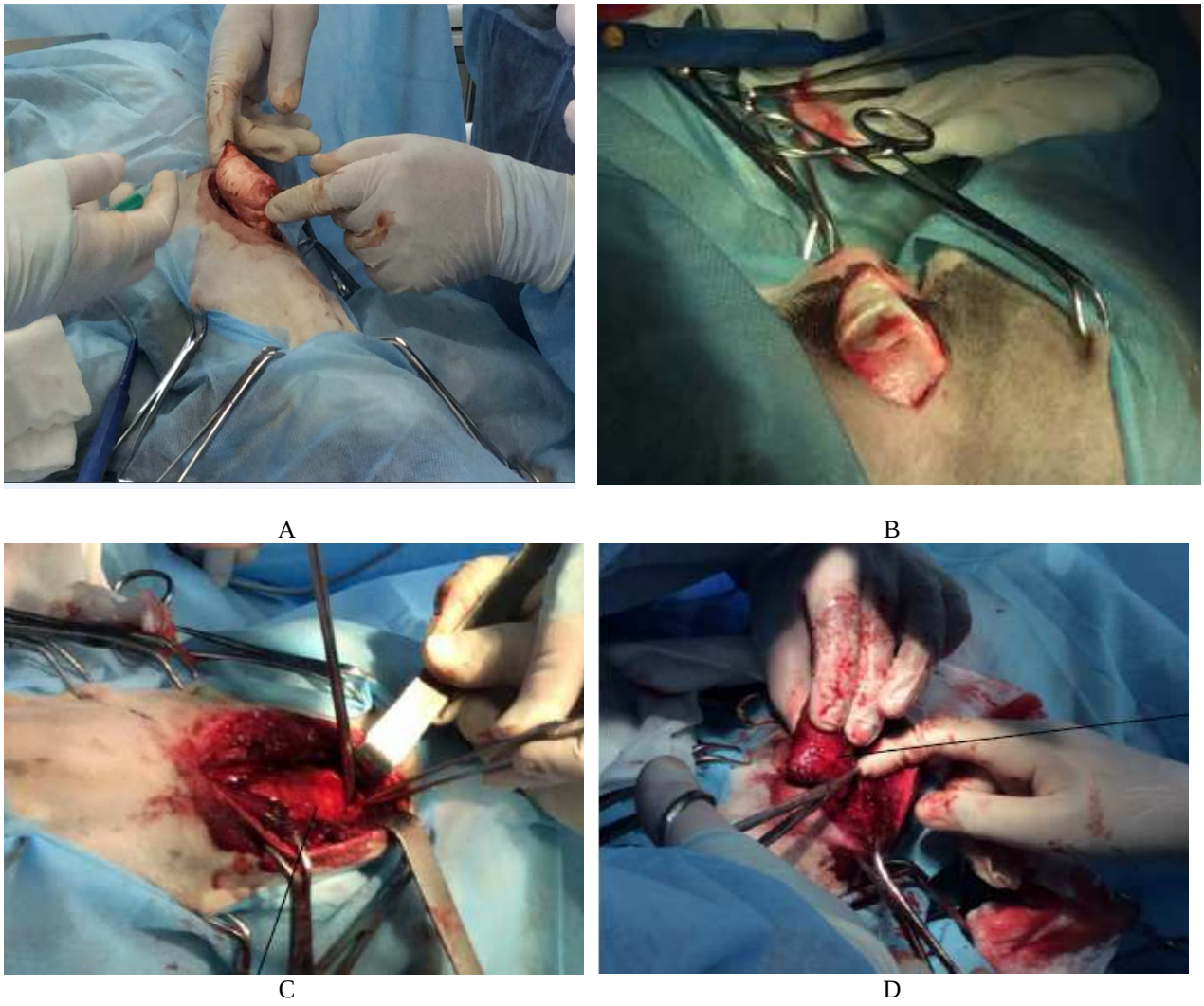
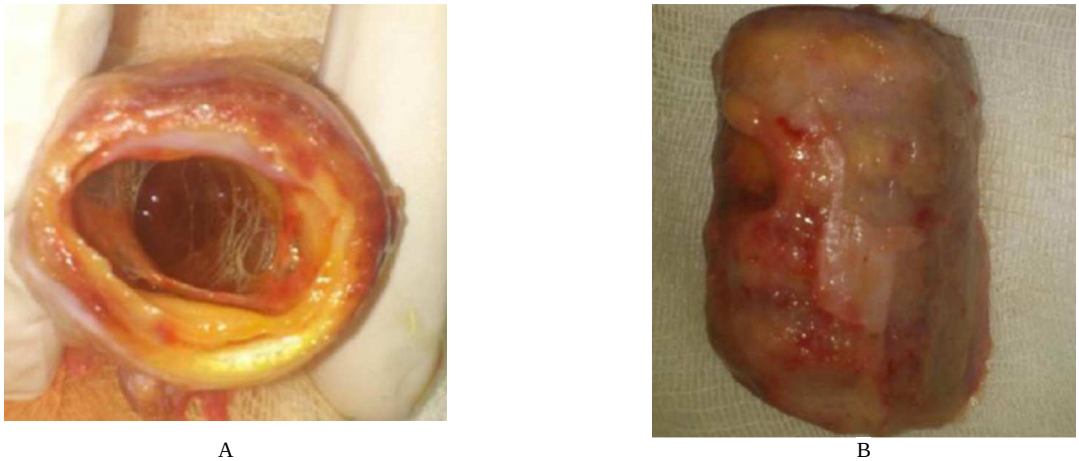


Figure 3: Main steps of heterotopic transplantation to dog and subsequent (after 1 months) removing of tracheal matrix sample colonized with MMSC

A – the formation of intramuscular pocket; B – implanted sample of tracheal matrix; C – isolation matrix from tissue; D – removing of matrix sample

In order to control the possible system reaction of transplant rejection or inflammation there was periodically carried out clinical blood test after heterotopic implantation of experimental samples to the dogs. The findings showed that during one month after implantation hematological parameters in animals-recipients ranged within the physiological norm. Visual inspection of the implantation area for the entire period of observation revealed no local rejection reactions: edema, festering, redness, appearance of fistulas, massive proliferation of fibrous tissue in the implant (Fig. 4).



A B

Figure 4: **Synthetic trachea matrix colonized with MMSC 30 days after heterotopic transplantation. General view of the synthetic tracheal matrix after removal from the body tissues of the recipient**
A – lumen of matrix, B – matrix covered with connective tissue capsule.

Excerpted from the groin 1 month after implantation tracheal matrices keep in shape, as well as properties of frame and was covered by connective tissue capsule intimately connected with matrix (Fig. 4). No inflammatory exudates or signs of inflammatory leukocyte infiltration were found during morphological examination. In the extracted trachea matrix at the same time preserved ultrafibrous structure was detected as well as strong colonization by recipient`s cells with formation of blood vessels and connective tissue fibers, extending from the surface into the implant (Fig. 5).

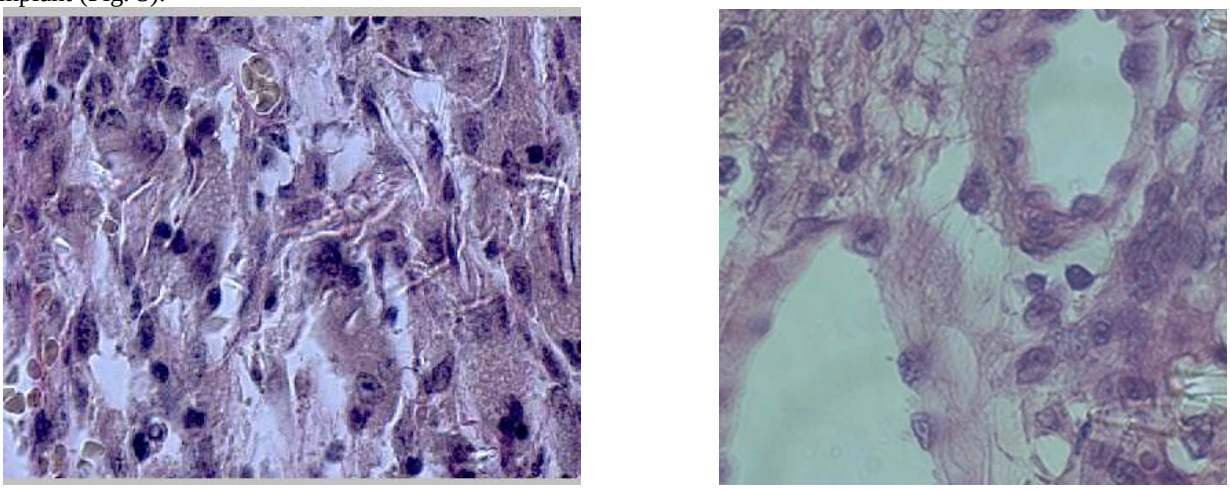


Fig. 5a **Fig. 5b**

Figure 5: **Active colonization of unwoven tracheal matrix by recipient`s cells**

In the last years, there is an increased interest for the researches dedicated to trachea transplantation and the main researches are concentrated on the creation of natural or synthetic material based 3D scaffolds. The most promising trend is creation of composite trachea matrix constructed of biocompatible porous and fibrous materials imitating native tissues which are colonized with recipient`s cells. In these hybrids (synthetic and biologic) matrixes or bioimplants optimal mechanical features and biocompatibility are combined [3, 5, 10]. Colonization of synthetic matrixes by tissue engineering prior to grafting, может in vitro (incubation with) or in vivo (heterotopic implantation, or in situ tissue engineering).We used two ways for obtaining tracheal bioimplant. In the beginning, synthetic matrix was colonized with recipient's MMSC ex vivo, after that there was heterotopic implantation. After 1 month, this allowed to get bioimplant based on synthetic scaffold with vascularized autologous connective tissue. Moreover, bioimplant didn`t cause any adverse effects like inflammation or rejection and saved its mechanical features. This way including preliminary prefabrication step of synthetic trachea matrix colonized with MMSC before grafting appears to be the most efficient.

CONCLUSION

The way of replacing trachea with trachea implant made from nonwoven trachea matrix colonized with recipient`s MMSC and following heterotopic implantation to recipient in order to get it vascularized and strongly colonized by native recipient`s cells seems to be one of the most promising. The main advantage of this method is natural formation of blood vessels so there is no inflammation and the implant is involved in the process of "homing" an thus fully gets a recipient`s morphology and becomes more biocompatible in the experiment then other existing variants.

Acknowledgement
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