Failure of airway healing in an ovine autotransplantation model that includes basic fibroblast growth factor

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Objective: Basic fibroblast growth factor is among the most potent promoters of angiogenesis. Its ability to enhance the blood supply to ischemic airways or nonvascularized tracheal autograft has been demonstrated. Its cumulative effect with muscular wrapping and its efficacy in a noncanine large animal model remain unknown. Treatment with basic fibroblast growth factor and muscular wrapping were compared with no special treatment and with muscular wrapping alone in an ovine tracheal autotransplantation model.

Methods: All sheep underwent orthotopic tracheal transplantation with 5 to 8 ring autografts in the cervical trachea. Fifteen sheep were classified randomly into the following three groups: no treatment (group A, n = 5), muscular wrapping with the right sternomastoid muscle (group B, n = 5), and topical administration of fibrin glue enriched with 2 $\mu\text{g}/\text{cm}^2$ basic fibroblast growth factor (group C, n = 5).

Results: Devascularized tracheal autografts were unable to maintain their structural integrity without other treatment (group A). However, the grafts were surrounded by well-vascularized connective tissue. In the muscular wrapping group (group B), infections occurred around the grafts, and the muscular wrapping was subject to necrosis. No neovascularization of the grafts occurred. Therapy with basic fibroblast growth factor (group C) led to improved muscular wrapping circulation and to adherence to the tracheal stumps. However, no success was achieved in validating the circulation in the grafts.

Conclusions: In contrast to the results achieved by other authors with canine models, the neovascularization of tracheal autografts was not achieved in sheep with the topical administration of basic fibroblast growth factor. Cranially pediculated muscular wrapping led to poorer circulation in the tissue around the graft than did no therapy at all.

Primary neoplasia, secondary tumors that have grown into the trachea, and severe tracheal trauma are rare but dangerous conditions. Dyspnea or hemorrhage may force the introduction of surgical therapy. In the event of long-segment resections in excess of 6 cm, tracheal transplantation would prove advantageous.¹ Nearly every type of material has been used for tracheal grafts. Three types of replacements can be found: prosthetic materials, organic grafts (both autografts and homografts), and combinations of both.² Experimental investigations in small test animals have

TABLE 1. Experimental design

Group	No.	Treatment of tracheal autografts
A (control)	5	None
B	5	Muscle wrapping
C	5	Muscle wrapping and local administration of bFGF

provided partially satisfactory results; as a result of the more rapid metabolism and better healing in these small animals, however, the techniques can be applied to large animals or even human beings only in a limited manner. Regardless of the method used to reduce the immunogenicity of allogeneic grafts, successful autotransplantation is a prerequisite for attempts at allogeneic transplantation. Successful tracheal autotransplantation in large animals has recently been reported by several study groups.^{3,4} The promotion of neo-vascularization with basic fibroblast growth factor (bFGF) appears particularly promising because it is both simple and safe. In comparison with such extensive surgical procedures as omentoplasty, laparotomy, and possibly even sternotomy, this treatment presents a clear advantage. bFGF is one of the most potent promoters of angiogenesis. It has been shown that bFGF may enhance the blood supply in rats,⁵ rabbits,⁶ and dogs.^{3,7}

Many investigations regarding tracheal transplantation have been carried out with canine models. In dogs, longitudinal vessels are present on the reverse of the trachea; such vessels are not present in the same way in human beings. In addition, dogs are proving increasingly difficult to obtain as study animals and are expensive to buy and look after. We recently reported on a large animal model involving sheep for tracheal surgery.⁸ This study was undertaken to assess the efficacy of human recombinant bFGF in an ovine tracheal autotransplantation model.

Material and Methods

Animals and Anesthesia

All operations were performed on 9-month-old female sheep weighing 40 to 50 kg. The study was approved by the animal care and use committees at the university and at the local district government (Ts No.: 509i-42502-00/288). The sheep were systemically healthy according to physical examination. Food was withheld for 24 hours. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and 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. Anesthesia was induced by intravenous injection of 0.2 mg/kg midazolam and 7 to 8 mg/kg propofol. The sheep were intubated with a cuffed endotracheal tube (inner diameter 9.0 mm, length 55 cm; Bivona Medical Technologies, Gary, Ind), and a stomach tube was inserted into the rumen. Anesthesia was maintained with isoflurane

at 2% to 2.5% end-tidal concentration in oxygen (2 L/min). In case of jet ventilation, anesthesia was maintained with intravenously administered propofol, and PO_2 and PCO_2 were measured continuously through an arterial catheter in the carotid artery (Paratrend7; Agilent Technologies, Palo Alto, Calif) (Figure 1, A and B). Ventilation was controlled to ensure normocapnia. For perioperative analgesia, 4 mg/kg carprofen was administered intravenously and 0.6 mg buprenorphine was administered intramuscularly. In addition, the surgical area was injected with 20 mL of lidocaine hydrochloride. Antibiotic therapy with sustained-release penicillin was started before the operation.

Surgical Technique of Tracheal Autotransplantation

The skin and subcutaneous tissues were incised laterally from 3 cm below the larynx, 12 cm in length. The cervical trachea was exposed by midline separation of the paired strap muscles and mobilized circumferentially for 8 cm. A 4-cm segment of the trachea, representing 5 to 8 cartilage rings, was removed. These autografts were placed in physiologic saline solution at room temperature for several minutes. Ventilation was continued after a spiral endotracheal tube was positioned in the lower trachea or by jet ventilation. The excised segment was then reimplanted in its original position as an autograft. The upper anastomosis was performed first with interrupted 4-0 polydioxanone sutures (Ethicon, Inc, Somerville, NJ). Full-thickness bites of all layers, usually around the adjacent cartilaginous rings, with sutures spaced 5 mm apart were used. Next, the membranous portion and two thirds of the cartilaginous portion of the lower anastomosis were sutured similarly. The spiral endotracheal tube or the jet catheter was then removed from the operative field, and ventilation was continued through the oral endotracheal tube. The lower anastomosis was subsequently completed.

Groups and Treatment of Autografts

The 15 study animals were classified randomly into 3 treatment groups (Table 1).

1. Group A ($n = 5$). No animal in group A underwent autograft therapy after the tracheal autotransplantation. The strap muscles were closed with a running polyglactin 910 suture (Vicryl; Ethicon); subcutaneous tissue and skin were sutured with 4-0 polyglactin 910 and 4-0 poliglecaprone 25 (Monocryl; Ethicon). The skin was sealed with a thiram spray (Nobecutane).

2. Group B ($n = 5$). Following tracheal autotransplantation, the right sternomastoid muscle was cranially pediculated. This pedicle was used to wrap the autograft circumferentially and encompassed it completely (Figure 1, C). The neck was closed in the same fashion as in group A.

3. Group C ($n = 5$). The same kind of pediculated sternomastoid muscle as was used in group B was prepared. Again, this muscle was wrapped around the autograft and fixed with sutures. Between the muscle and the autograft, topical fibrin glue with bFGF was administered. The effectivity and optimal dosage of bFGF in fibrin glue have been extensively examined in previous studies.^{3,5-7} A 2-mL portion of fibrin glue (Beriplast P; Aventis Behring GmbH, Marburg, Germany) consisting of 1 mL fibrinogen and aprotinin and 1 mL thrombin and calcium chloride was mixed with 50 μ g bFGF (Strathmann Biotec AG, Hamburg, Germany) and distributed between the muscle and the autograft and covering

TABLE 2. Survival and graft status

Sequential No.	Scheduled survival (d)	Achieved survival (d)	Mean survival of sheep dead before scheduled date (d)	Graft status at time of death (macroscopic aspect)
Group A (control)			18.6	
1	7	9		Mucosal layer intact, cartilage intact
2	14	14		Mucosal necrosis, cartilage intact
3	28	18		Airway stenosis, necrosis
4	56	16		Airway stenosis, necrosis
5	180	22		Mucosal layer intact, abscess with cartilage collapse
Group B (muscle wrapping)			12.8	
1	7	6		Mucosal necrosis, abscess
2	14	14		Mucosal necrosis, abscess perforation
3	28	15		Dissolution, severe airway stenosis
4	56	9		Mucosal necrosis, abscess
5	180	13*		Mucosal necrosis, abscess
Group C (wrapping plus bFGF)			12.6	
1	7	6		Mucosal layer intact, cartilage intact
2	14	9		Mucosal dissolution, cartilage collapse
3	28	15		Airway stenosis, necrosis
4	56	13*		Mucosal dissolution, cartilage collapse
5	180	10		Mucosal necrosis, cartilage intact

For all groups, n = 5.

*Died of sudden respiratory failure.

the anastomosis region. The dose of bFGF used is comparable to 2 $\mu\text{g}/\text{cm}^2$ (according to a formula described by Nakanishi and colleagues.³ A 1-mL portion of thrombin and calcium chloride (Beriplast P) was used to reconstitute the lyophilized bFGF. This mixed solution and 1 mL fibrinogen and aprotinin (Beriplast P) were administered to the graft simultaneously. The outer surfaces of the autografts, including the anastomoses, were soaked circumferentially with the drugs. Strap muscles, subcutaneous tissues, and skin were closed as described for group A.

Postoperative Management

The animals were given preoperative antibiotic coverage (intramuscular injection of sustained-release penicillin as 1.2 million IU benzylpenicillin-benzathine plus 300,000 IU benzylpenicillin-procaine; Tardomyocel; Bayer AG, Leverkusen, Germany) and received a second antibiotic dose after 48 hours. The sheep were observed daily for coughing and dyspnea. Coughing was recorded as absent, as mild if the sheep rarely coughed, as moderate if the sheep coughed intermittently, or as severe if the sheep coughed continuously. Dyspnea was recorded as present or not present. The cervical incisions were evaluated daily for swelling, inflammation, seroma formation, subcutaneous emphysema, and signs of infection.

Necropsy

All sheep were killed with an overdose of sodium pentobarbital (Eutha77; Aventis Pharma AG, Frankfurt am Main, Germany) according to the planned survival time or because of clinical evidence of severe dyspnea or local infection. Planned postoperative survival times in each group were 1 week, 2 weeks, 4 weeks, 8 weeks, and 6 months. With the pentobarbital, 25,000 IU heparin

sodium was administered into a central vein. Autopsy was performed immediately after the animal's death. Barium sulfate at 50 mL (120% weight/volume, Micropaque; Guerbet GmbH, Sulzbach, Germany) was injected into the bilateral carotid arteries. After the tracheal microvessels were filled with the barium sulfate solution, the tracheal graft, including both anastomotic sites, was removed. The entire trachea, with the larynx, the grafts, and both anastomotic sites, was removed. Abnormal necropsy findings were recorded. The specimens were fixed with 10% natural buffered formalin for at least 48 hours. After fixation, xeroradiography of the graft was performed to obtain the microangiogram. All the fixed grafts were cut longitudinally at the anterior wall, embedded in paraffin, longitudinally sectioned, and stained with hematoxylin-eosin, Alcian blue, and periodic acid-Schiff. The viability of the autografts was evaluated by epithelial morphologic examination and the assessment of submucosal vessels. The percentages of epithelial, cartilage, and peritracheal tissues contained were documented. The percentage of necrotic tissue was also determined.

Results

Survival

All sheep survived the operation without any problems. In the control group (group A, no muscular wrapping and no bFGF), the animals that were intended to survive for 1 week (9 days) and 2 weeks were killed according to schedule. The other animals, which were intended to survive for longer periods, had to be killed on postoperative days 16, 18, and 22 because of dyspnea and audible respiratory sounds. Table 2 reveals a detailed list.

In the group with muscular wrapping (group B), the first

2 animals, with planned survival times of 1 and 2 weeks, were also killed according to schedule. The other animals had to be killed on postoperative days 9 and 15 because of dyspnea and audible respiratory sounds. One animal died on day 13.

In the group with muscular wrapping and local bFGF administration (group C), only the first animal was killed according to schedule after 1 week. The other animals had to be killed on postoperative days 9 and 13 because of dyspnea and audible respiratory sounds. The remaining 2 animals were then killed on postoperative days 10 and 13 without any clinical indications of dyspnea, because no further successful attempt was expected in this case.

Macroscopic Condition of the Grafts

1. Group A. In the animal that was killed after 1 week, the graft was securely anchored to the recipient trachea with its sutures. The rows of sutures were watertight and airtight. However, the graft had not been integrated by the body in any way. The peritracheal tissue, which had remained on the graft's cartilaginous braces, had perished but not been removed. The graft's mucous membrane was pale but shiny and without defects (Figure 2, A). After 14 days the mucous membrane was dark and livid and had already become detached in a few small areas. The tracheal graft was still firmly anchored but had not adhered to the surrounding tissue (Figure 2, B). The actual graft was firmly encapsulated by a ring of firm connective tissue. The findings in those animals that were killed during the further course (postoperative days 16, 18, and 22) were similar. The graft was encapsulated by firm connective tissue. With time, however, this connective tissue again became soft, so that it was able to give during inhalation. The mucous membrane had perished, and the cartilage had begun to shrink and was soft (Figure 2, C). This remodeling led to the increasing stenosis of the respiratory tract during inhalation.

2. Group B. In the animal with a survival time of 1 week, the transplant site was infected. The muscle wrapped around the graft was predominantly necrotic. No connection had been achieved between the graft and the muscle. The graft's mucous membrane had perished (Figure 2, D). In the case of the other animals, which had to be killed on days 13, 14, and 15, progressive necrosis of the mucous membrane, which was partially excoriated, was revealed (Figure 2, E). The cartilage was soft and also infected. The transplant site was revealed to be severely infected, and the muscular wrapping was necrotic.

3. Group C. After 1 week the mucous membrane and the cartilage in this group was revealed to be macroscopically intact. The transplant site was not infected. The row of sutures was impermeable. Part of the muscle had adhered to the graft. In the next 2 animals, on which necropsies were performed because of dyspnea 9 and 13 days after transplantation, the mucosa had perished and was partially per-

forated, and the cartilage had broken down (Figure 2, F). Although the muscle was also partially attached to the graft in this case no visible neovascularization had been achieved. In contrast, the muscle at the nontransplanted ends of the trachea had become firmly integrated. The graft itself was surrounded by infected fluid. The last 2 animals were then removed from the study. In each of these animals, the graft's mucous membrane had perished and the cartilage was still predominantly covered. The infection at the transplant site was locally delimited; the muscular wrapping was partially intact. The cartilage had not yet become softened (Figure 2, G).

Histologic Examination

Examination with an optical microscope predominantly confirmed the findings of the macroscopic autopsy. The details are summarized in Table 3 and Figure 1, D to G. In the final analysis, the tracheal mucous membrane was necrotic in all animals and at all points in time. Differences were merely in the degree of desquamation and progressive destruction. The same was revealed in the case of the peritracheal tissue. This was also necrotic in all animals and at all points in time. The differences with regard to decomposition were significantly dependent on the existence of a peritracheal infection. In the cartilage, the percentage of necrosis was determined according to the number of vital cell nuclei. Clear differences were revealed in this case, with worse results for pure muscular wrapping, especially in favor of those animals treated with bFGF.

Microangiography

In group A, the circulation of the connecting tissue around the tracheal graft was shown to increase with time (Figure 3, A). Although the circular, intact preparation (Figure 3, A, after 14 days) gives that impression, the graft itself is not supplied with blood. Figure 3, B, reveals this in the dissected preparation. Only Figure 3, C, after 22 days, reveals a cloudy contrast at the edge of the graft. In macroscopic terms, however, this finding did not correspond to circulation, but rather to the leakage of contrast agent at the edge of the healthy mucous membrane.

In group B, circulation around the grafts was revealed to be considerably poorer. As a result of the infected and partially necrotic muscular wrapping, no relevant circulation was achieved in and around the grafts (Figure 3, D and E).

Group C also did not reveal any graft circulation. Only an improvement in vascularization at the tracheal stumps was determined (Figure 3, F). The muscular wrapping was firmly integrated and vascularized in this location. In the case of advanced infection around the graft (as in Figure 3, G), this effect could no longer be detected.

Figure 1. Intraoperative situation and histologic examination. **A,** Intraoperative situation during autotransplantation. Posterior wall is sutured for both proximal and caudal anastomoses. Jet catheter is inserted into distal trachea for ventilation. Catheter is inserted through right carotid artery for continuous monitoring of PO_2 , PCO_2 , pH, and saturation. **B,** Anastomosis is completed. Arterial cannula is still in carotid artery. **C,** After anastomosis, muscular wrapping is carried out with cranially pediculated right sternomastoid muscle. This is dorsally guided to left to trachea at rear of graft, is wrapped around trachea, and is loosely affixed to itself and to trachea. **D,** Optical micrograph of autograft after 14 days (group A; hematoxylin and eosin stain, original magnification $\times 25$). Cartilage in left half of figure belongs to native trachea; cartilage on right belongs to autograft. Mucous membrane of native trachea, coming from left, slowly makes transition from columnar epithelium to flat metaplasia, which is seated on inflamed tissue. This pseudomucosa slightly covers exposed graft cartilage on right of figure. Actual mucous membrane of graft cartilage has already completely disappeared. **E,** Autograft with muscular wrapping after 6 days (group B; hematoxylin and eosin stain, original magnification $\times 25$). Mucous membrane is still present, as is submucosal tissue, but both are necrotic. Both cartilages belong to graft, and already reveal extensive necrosis, particularly in comparison with Figure 1, **D**. **F,** Autograft with muscular wrapping and bFGF after 10 days (group C; hematoxylin and eosin stain, original magnification $\times 25$). Mucosa is also necrotic but still present in this case. More cartilage is still vital than in Figure 1, **E**. **G,** Same preparation as in Figure 1, **F** (hematoxylin and eosin stain, original magnification $\times 25$). Left cartilage belongs to native trachea; right cartilage belongs to autograft. In this case mucous membrane also covers inflamed tissue in direction of graft but does not yet reach gap between two cartilages at this time.

Figure 2. Macroscopic aspect of grafts. A, Autograft 9 days after transplantation. Rows of sutures and mucous membrane are intact. Graft's mucous membrane is not supplied with blood. B, Autograft after 16 days. Necrosis of graft's mucous membrane is now clearly visible. However, mucous membrane is still complete and covers cartilaginous braces. Mucous membrane is beginning to retract at tracheal stumps. C, Autograft after 22 days. Because of cicatricial shrinkage, graft is increasingly being pushed into lumen. Mucous membrane is not supplied with blood. Cartilage is beginning to break down. D, Autograft with muscular wrapping after 1 week. Mucous membrane is not supplied with blood. Rows of sutures are still intact. Graft's mucous membrane is slowly being absorbed at edge. E, Autograft with muscular wrapping after 15 days. Graft's mucous membrane is necrotic and already partially detached. Healthy mucous membrane is beginning to retract at tracheal stumps, so healthy cartilaginous braces are also being exposed. Graft's cartilage is softened, necrotic, and collapsing into lumen; catastrophic findings. F, Autograft with muscular wrapping and bFGF on day 9. Graft's mucous membrane is necrotic and reveals minor perforations. Rows of sutures are still intact. G, Autograft with muscular wrapping and bFGF on day 10 (before scheduled date). Commencement of cicatricial retraction. Stump mucous membrane is also retracted. Graft's mucous membrane is dark, indicating progressive necrosis.

TABLE 3. Histologically determined degree of necrosis and cell loss

Sequential No.	Survival (d)	Premature termination	Epithelial necrosis (%)	Desquamation (%)	Cartilage necrosis (%)	Dissolution (%)	Peritracheal graft tissue necrosis (%)	Dissolution (%)
Group A			100				100	
	9			0	50	0		0
	14			0	40	0		0
	18	Yes		80	70	0		80
	16	Yes		100	60	80		100
Group B	22	Yes		80	30	100		50
			100				100	
	6			50	40	0		50
	9	Yes		80	30	0		0
	15	Yes		100	90	50		100
Group C	14	Yes		100	90	70		100
	13	Yes		50	80	50		50
			100				100	
	6			0	20	0		0
	9	Yes		50	50	50		50
	15	Yes*		20	30	0		20
	13	Yes		70	70	50		30
	10	Yes*		20	20	0		50

*Premature termination of study because a positive outcome was no longer to be expected.

Discussion

The replacement of the trachea by transplantation remains an unsolved problem.^{9,10} With few exceptions, clinical attempts at allogeneic or alloplastic replacement have remained unsuccessful to date. The main problems in the case of allogeneic transplantation are the supply of blood to the graft and immunogenicity. Whereas autografts in small animals are able to heal even without the introduction of additional measures, this does not apply to large animals or human beings.¹¹⁻¹³ Various procedures for restoring circulation as rapidly as possible are described. Single-stage and multistage procedures compete with one another in this case.^{14,15} Successful procedures involving muscular wrapping^{3,16} and the greater omentum^{14,17} have been described. Cryopreservation,^{12,13,18-20} detergents,²¹ and immunosuppression²² compete as methods of reducing immunogenicity.

Although muscular wrapping and omental grafting have been reported as being successful, there is not under any circumstances any certain guarantee of successful neovascularization. Intensive research into further measures is therefore being done. One approach is an attempt to directly promote angiogenesis. Various vascular growth factors, the efficacies of which have been verified, have been isolated. Among the angiogenesis factors best investigated to date is bFGF, a protein with a molecular weight of 17 kd and an efficacy shown both *in vitro* and *in vivo*.²³⁻²⁷ Soon after its discovery, bFGF was being used in the revascularization of tracheal grafts.^{5,6,28}

A report regarding the improvement in tracheal autotransplantation healing in dogs with bFGF was recently published.³ In this report, the treatment of the autografts with bFGF was equivalent to pediculated muscular wrapping or omental grafting. We attempted to reproduce these results in a cervical autotransplantation model in sheep. On the basis of the data at hand,³ a group with bFGF without muscular wrapping was not used. Rather, our goal was to ascertain whether the effects of bFGF and muscular wrapping were additive or increasing. A negative effect from the muscle wrapping was not expected.

Cervical autotransplantation without any additional measure revealed astonishingly good results. A longer survival time (18.6 days) was achieved than with either muscular wrapping alone or bFGF with muscular wrapping. The grafts were ensheathed by firm connective tissue. Good vascularization of the connective tissue was also revealed, but no relevant assumption of contact with the grafts occurred, with the results that this tissue became necrotic, shrank, and finally prolapsed into the lumen. In comparison with the untreated grafts, simple muscular wrapping with the sternomastoid muscle was revealed to be inferior. This result was initially astonishing. Although the muscle was vital during the operation, certain sections appeared to be-

come necrotic afterward and to become infected through the obligatorily contaminated trachea. This infection then led to the total necrosis of the muscle and prevented any neovascularization of the graft. The situation did not improve considerably when bFGF was administered. In this case the muscle healed well and firmly with the cranial and caudal tracheal stumps. However, no considerable graft neovascularization was achieved.

The results clearly show that a unilaterally pediculated

Figure 3. Microangiography of tracheal grafts. A, Autograft 14 days after transplantation. Trachea has remained circularly intact. Strong intercartilaginous arteries at intact edges of trachea can clearly be seen. In area of graft, however, vessels are present only in surrounding connective tissue. Circulation in graft is uncertain. B, Autograft 16 days after transplantation. Also good depiction of tracheal stumps in this case. Graft has shrunk in longitudinally and transversely. Circulation depicted in area of graft is attributable to peritracheal connective tissue. C, Autograft 22 days after transplantation. Good circulation is seen in tracheal stumps and around graft. Graft itself is not supplied with blood. D, Autograft with muscular wrapping (group B) after 15 days. Circulation is present only in healthy trachea. No circulation can be detected in graft or, because of infected muscular wrapping, in peritracheal tissue. E, Autograft with muscular wrapping (group B) after 14 days. Again, no circulation is present in either graft or surrounding connective tissue. F, Autograft with muscular wrapping and bFGF (group C) 7 days after transplantation. Good tracheal stump circulation is present. Particularly on left (arrow), circulation in attached cicatricial tissue on tracheal stump is also clearly visible. No blood is being supplied to muscular wrapping or graft. G, Autograft with muscular wrapping and bFGF (group C) after 9 days. Good stump circulation is present. No blood is being supplied to graft or muscle.

sternomastoid muscle is not sufficient to vascularize a cervical tracheal autotransplantation. Rather, the muscular wrapping opens the area to extensive infection. This cannot be compensated for by the positive effect of the bFGF. Whether the almost obligatory pulmonary contamination of the sheep plays a role in this remains unclear. The reason that certain study groups required extensive intervention to achieve the same purpose^{3,7,14,16,17} also remains unclear. The revascularization of the intrinsic transverse intercartilaginous arteries after 7 days appears improbable not only for the specific situation of the trachea but also for any other type of nonvascularized autograft.⁴

Because of the published data,³ we had expected to achieve an improvement in neovascularization with bFGF. Contrary to our expectations, the extended preparation of the cervical muscles with a pediculated sternomastoid muscle led to different results. At the same time, the graft and the muscular wrapping became infected through the trachea. A subjective advantage was perceived for the animals treated with bFGF, but this was difficult to objectify on the basis of the data presented here.

We have drawn several conclusions from these studies. In sheep, cervical autografts without any further measures are ensheathed in well-vascularized connective tissue, but neovascularization is not achieved before the grafts become necrotic and relocate the respiratory tract. Unilaterally pediculated muscular wrapping does not promote the circulation sufficiently to facilitate autotransplantation but may provide a nutritive medium for extensive infections. Addition of bFGF in fibrin glue considerably improves adhesion and the cicatricial healing of muscular wrapping, at least in healthy tracheas.

Our investigation clearly revealed that the results obtained from a given large animal model cannot necessarily also be transposed to another large animal model.³ There is no ideal large animal model for tracheal surgery. Individual animals correspond better or less well to human beings with regard to individual characteristics. It would therefore appear sensible to check initially successful experimental investigations with different large animal models before initiating clinical studies.

On the basis of these conclusions, we are planning a similar series of studies in which the respiratory tract will be temporarily splinted (stented) to allow additional time for the grafts to heal. At the same time we plan to attempt to bring the cervical muscles to the graft without pediculating them over long segments. The purportedly positive results with bFGF also lead us to continue to attempt to promote neovascularization with this agent. The distant aim of our continued investigations remains the cervical transplantation of primarily nonvascularized, allogeneic tracheas. We continue to search for a procedure that can be implemented practically without omentoplasty or laborious plastic sur-

gery. Currently, however, we still lack a large animal model in which autograft healing can be achieved in a reliable and reproducible manner.

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References

1. Grillo HC. Reconstruction of the trachea: experience in 100 consecutive cases. *Thorax*. 1973;28:667-79.
2. Grillo HC. Surgery of the trachea. *Curr Probl Surg*. 1970;3:59.
3. Nakanishi R, Hashimoto M, Yasumoto K. Improved airway healing using basic fibroblast growth factor in a canine tracheal autotransplantation model. *Ann Surg*. 1998;227:446-54.
4. Moriyama H, Sasajima T, Hirata S, Yamazaki K, Yatsuyanagi E, Kubo Y. Revascularization of canine cryopreserved tracheal allografts. *Ann Thorac Surg*. 2000;69:1701-6.
5. Mayer E, Cardoso PF, Puskas JD, De Campos K, Oka T, Dardick I. The effect of basic fibroblast growth factor and omentopexy on revascularization and epithelial regeneration of heterotopic rat tracheal isografts. *J Thorac Cardiovasc Surg*. 1992;104:180-8.
6. Albes JM, Klenzner T, Kotzerke J, Thiedemann KU, Schafers HJ, Borst HG. Improvement of tracheal autograft revascularization by means of fibroblast growth factor. *Ann Thorac Surg*. 1994;57:444-9.
7. Nakanishi R, Nagaya N, Yoshimatsu T, Hanagiri T, Yasumoto K. Optimal dose of basic fibroblast growth factor for long-segment orthotopic tracheal autografts. *J Thorac Cardiovasc Surg*. 1997;113:26-36.
8. Behrend M, Klempnauer J. Influence of suture material and technique on end-to-end reconstruction in tracheal surgery: an experimental study in sheep. *Eur Surg Res*. 2001;33:210-6.
9. Macchiarini P. Tracheal transplantation: beyond the replacement of a simple conduit [editorial]. *Eur J Cardiothorac Surg*. 1998;14:621-3.
10. Grillo HC. Tracheal replacement [editorial]. *Ann Thorac Surg*. 1990;49:864-5.
11. Kim KH, Sung MW, Chang KH, Kang BS. Therapeutic dilemmas in the management of thyroid cancer with laryngotracheal involvement. *Otolaryngol Head Neck Surg*. 2000;122:763-7.
12. Carbognani P, Spaggiari L, Solli P, Corradi A, Cantoni AM, Barocelli E. Experimental tracheal transplantation using a cryopreserved aortic allograft. *Eur Surg Res*. 1999;31:210-5.
13. Nakanishi R, Hashimoto M, Muranaka H, Umesue M, Kohno H, Yasumoto K. Maximal period of cryopreservation with the Bicell biofreezing vessel for rat tracheal isografts. *J Thorac Cardiovasc Surg*. 1999;117:1070-6.
14. Li J, Xu P, Chen H. Successful tracheal autotransplantation with two-stage approach using the greater omentum. *Ann Thorac Surg*. 1997;64:199-202.
15. Hardillo JA, Vander Poorten V, Delaere PR. Transplantation of tracheal autografts: is a two-stage procedure necessary? *Acta Otorhinolaryngol Belg*. 2000;54:13-21.
16. Mello-Filho FV, Mamede RC, Velludo MA. Tracheal neovascularization: a method involving mobilization of a complete tracheal neovascularized segment using a sternohyoid muscle flap. *Laryngoscope*. 1996;106(1 Pt 1):81-5.
17. Murai K, Oizumi H, Masaoka T, Fujishima T, Abiko M, Shiono S. Removal of cartilage rings of the graft and omentopexy for extended tracheal autotransplantation. *Ann Thorac Surg*. 1999;67:776-80.
18. Mukaída T, Shimizu N, Aoe M, Andou A, Date H, Okabe K. Experimental study of tracheal allotransplantation with cryopreserved grafts. *J Thorac Cardiovasc Surg*. 1998;116:262-6.
19. Aoki T, Yamato Y, Tsuchida M, Souma T, Yoshiya K, Watanabe T. Successful tracheal transplantation using cryopreserved allografts in a rat model. *Eur J Cardiothorac Surg*. 1999;16:169-73.

20. Yokomise H, Inui K, Wada H, Ueda M, Hitomi S. Long-term cryopreservation can prevent rejection of canine tracheal allografts with preservation of graft viability. *J Thorac Cardiovasc Surg.* 1996;111:930-4.
21. Liu Y, Nakamura T, Yamamoto Y, Matsumoto K, Sekine T, Ueda H. Immunosuppressant-free allotransplantation of the trachea: the antigenicity of tracheal grafts can be reduced by removing the epithelium and mixed glands from the graft by detergent treatment. *J Thorac Cardiovasc Surg.* 2000;120:108-14.
22. Hashimoto M, Nakanishi R, Muranaka H, Umesue M, Eifuku R, Yasumoto K. Short-course immunosuppression using FK506 for rat tracheal allografts. *J Cardiovasc Surg (Torino).* 2000;41:487-92.
23. Folkman J, Klagsbrun M. Angiogenic factors. *Science.* 1987;235:442-7.
24. Gospodarowicz D. Localisation of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth. *Nature.* 1974;249:123-7.
25. Gospodarowicz D, Baird A, Cheng J, Lui GM, Esch F, Bohlen P. Isolation of fibroblast growth factor from bovine adrenal gland: physicochemical and biological characterization. *Endocrinology.* 1986;118:82-90.
26. Thompson JA, Anderson KD, DiPietro JM, Zwiebel JA, Zametta M, Anderson WF. Site-directed neovessel formation in vivo. *Science.* 1988;241:1349-52.
27. Eppley BL, Doucet M, Connolly DT, Feder J. Enhancement of angiogenesis by bFGF in mandibular bone graft healing in the rabbit. *J Oral Maxillofac Surg.* 1988;46:391-8.
28. Olech VM, Keshavjee SH, Chamberlain DW, Slutsky AS, Patterson GA. Role of basic fibroblast growth factor in revascularization of rabbit tracheal autografts. *Ann Thorac Surg.* 1991;52:258-64.