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EXPERIMENTAL STUDY

Comparison of different methods of circumferential tracheal reconstruction: an experimental study

Sirküler trakeal rekonstrüksiyonda farklı yöntemlerin karşılaştırılması: Deneysel çalışma

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Objectives: This study was designed to compare different prefabrication models for circumferential tracheal reconstruction.

Materials and Methods: Thirty adult female New Zealand rabbits were divided into six groups for circumferential tracheal reconstruction with a different tissue combination. Prefabrication of neotrachea was performed without anastomosis. Lateral thoracic fascia or pectoralis major muscle were used for vascular supply. Polypropylene mesh, polytetrafluoroethylene (Gore-Tex), or cartilage rings were used for skeletal framework. Hairless epithelial skin graft was used for inner lining. The groups were compared in terms of viability of hairless epithelial graft, longitudinal flexibility, rigidity, flap wall thickness, internal diameter, and flap viability.

Results: Epithelial skin graft was almost totally viable (95%-99%) in the polypropylene mesh groups. Gore-Tex groups exhibited almost total necrosis (74%-71%). Longitudinal flexibility and rigidity were similar to the native trachea in Gore-Tex and polypropylene mesh groups. Neotrachea prefabricated with cartilage grafts showed more than normal longitudinal flexibility and collapsed easily.

Conclusion: The prefabricated model with the use of polypropylene mesh for skeletal framework and lateral thoracic fascia for vascular supply seems to be the best alternative in the reconstruction of circumferential tracheal defects.

Key Words: Bioprosthesis; epithelium/transplantation; graft survival; rabbits; surgical flaps; trachea/surgery.

Amaç: Bu çalışmada sirküler trakeal rekonstrüksiyon için farklı prefabrike modellerin karşılaştırılması amaçlandı.

Gereç ve Yöntem: Otuz adet erişkin dişi Yeni Zelanda tavşanı, farklı doku kombinasyonuyla sirküler trakeal rekonstrüksiyon için altı gruba ayrıldı. Prefabrike edilmiş trakealarda anastomoz yapılmadı. Vasküler kaynak olarak lateral torasik fasya veya pektoralis majör kası; iskeletsel çatı olarak polipropilen mesh, politetrafloroetilen (Gore-Tex) veya yüzük kartilajlar kullanıldı. İç yüzey tüysüz epitelyal deri greftinden sağlandı. Gruplar tüysüz epitel greftinin canlılığı, longitüdinal esneklik, dayanıklılık, flep duvar kalınlığı, iç çap ve flep canlılığı açısından karşılaştırıldı.

Bulgular: Polipropilen mesh gruplarında tama yakın epitelyal deri grefti canlılığı görülürken (%95-%99), Gore-Tex gruplarında tama yakın nekroz gözlendi (%74-%71). Longitüdinal esneklik ve dayanıklılık Gore-Tex ve polipropilen mesh gruplarında normal trakeaya yakın ölçülerde bulundu. Kartilaj greftleri ile prefabrikasyon yapılan gruplar normalden fazla longitüdinal esneklik ve kolayca kollaps gösterdi.

Sonuç: İskeletsel çatı olarak polipropilen mesh, vasküler kaynak olarak lateral torasik fasyanın kullanıldığı prefabrike model, sirküler trakea defektlerinin rekonstrüksiyonunda en iyi seçenek olarak görünmektedir.

Anahtar Sözcükler: Biyoprotez; epitel/transplantasyon; greft canlılığı; tavşan; cerrahi flep; trakea/cerrahi.

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The trachea has a highly specialized structure owing to the combination of its tissue and mechanical properties. Oncologic, traumatic, and thermal injuries may cause circumferential or noncircumferential tracheal defects. The procedure to be performed for reconstruction of circumferential tracheal defects depends on the size of the defect. Primary end-to-end anastomosis without excessive tension is the aim of the operation.[1] With extensive mobilization of the stumps, 50 percent of tracheal length can be resected and reconstructed by primary end-to-end anastomosis. But circumferential defects longer than 5-6 cm or more than half of the total tracheal length can only be reconstructed by using flaps or synthetic grafts.[2-4]

In optimal tracheal reconstruction, the substitute for bridging the defect must be airtight, rigid enough to avoid collapse, longitudinally flexible, lined by an epithelial layer, and finally well-vascularized to avoid infections and to provide acceptable wound healing. Therefore, the substitute should have three basic components: an inner epithelial lining, a skeletal framework, and a rich blood supply. However, such a substitute has not been introduced yet and all the alternative methods are unsatisfactory in terms of one or two of the basic components.

This experimental study was designed to compare prefabricated flap models with different tissue combinations in order to substitute three basic components of the trachea in reconstruction of circumferential defects: respiratory epithelium (inner lining), tracheal cartilage (skeletal framework), and tracheal adventitia (vascular supply).

MATERIAL AND METHODS

Thirty adult female New Zealand rabbits weighing 3 to 3.5 kg were used. All operations were performed under intramuscular ketamine hydrochloride (50 mg/kg) and xylazin hydrochloride (5 mg/kg) anesthesia. All procedures were performed under sterile conditions and in accordance with the Helsinki declaration. The rabbits were divided into six groups and a different tissue combination was used in each group for substitution of three basic components of the trachea.

Substitution of inner lining

In all the groups, hairless epithelial graft obtained from the inner surface of rabbit ear was used to replace respiratory tract epithelium.

Substitution of skeletal framework

In the first and second groups, four units of cartilage rings which had 2 mm width and 15 mm length were used. The third and fourth groups received polytetrafluoroethylene (Gore-Tex) carotid vessel grafts with a diameter of 6 mm. In the fifth and sixth groups, polypropylene mesh was used.

Substitution of vascular supply

Tracheal adventitia was replaced with pectoralis major muscle in the first, third, and fifth groups, and with lateral thoracic fascia in the remaining groups.

The tissue combinations of the groups are shown in Table I.

Surgical technique

To obtain hairless epithelial lining; a full-thickness skin graft, 3x3 cm in size, was harvested from the most proximal part of the inner surface of the external ear. The defect was left to secondary healing which took three weeks. The epithelium covering the defect was hairless (Fig. 1).

In group 1, the cartilage block graft was taken from the ear and split into four strands that were 2x15 mm in size (Fig. 2). The hairless epithelial graft was tubed around a 16-F urinary silicone catheter so that its epidermal side faced the catheter and then

TABLE I

COMBINATIONS OF AUTOLOGOUS AND ALLOPLASTIC MATERIALS IN SIX GROUPS

Component	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Inner lining	HEG	HEG	HEG	HEG	HEG	HEG
Skeletal framework	CRG	CRG	GCVG	GCVG	PM	PM
Vascular supply	PMM	LTF	PMM	LTF	PMM	LTF

HEG: Hairless epithelial graft; CRG: Cartilage ring graft; GCVG: Gore-Tex carotid vessel graft; PM: Polypropylene mesh; PMM: Pectoralis major muscle; LTF: Lateral thoracic fascia.

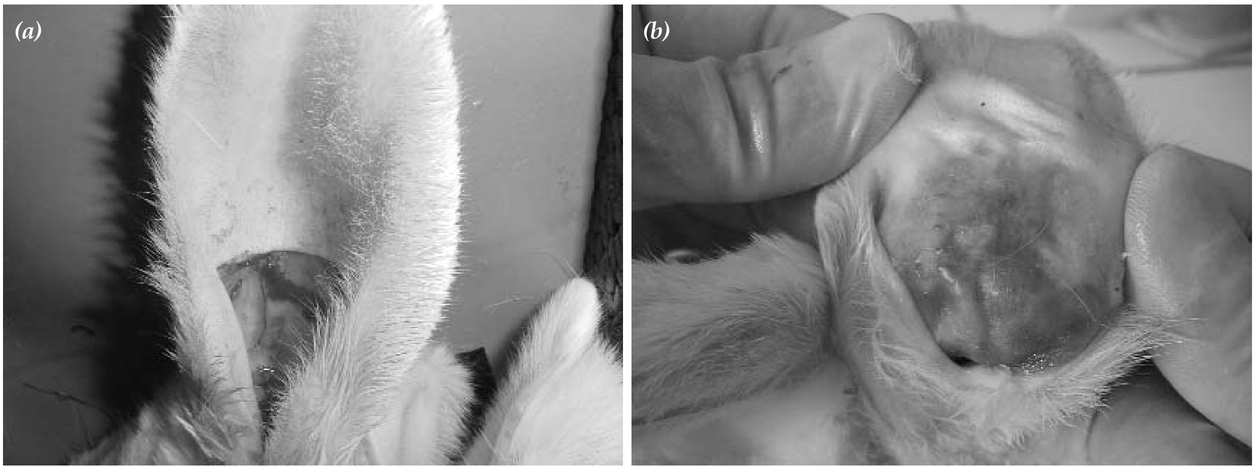

Fig. 1 - ***(a)*** *A full-thickness skin defect, 3x3 cm in size, is formed.* ***(b)*** *The defect is left to secondary healing for re-epithelization by hairless epithelium.*

cartilage strands were ringed around the dermal sides of the hairless epithelial graft.

The edges of the cartilage strands were sutured with 5/0 polypropylene suture material. By using a horizontal midthoracic incision, pectoralis major muscle was reached. Silicone catheter, hairless epithelial graft, and cartilage rings were wrapped with pectoralis major muscle (Fig. 3). The skin incision was sutured with polypropylene 3/0 suture material.

In group 2, the surgical technique was similar to the first group. But in this group, lateral thoracic fascia was used for vascular supply. The reflection of the lateral thoracic artery can be inspected externally in the lateral thorax and abdomen. The incisions were planned medially to the pedicle. Skin incisions were made starting from the xiphoid, advancing laterally towards the distal abdominal portion and the skin was dissected from the fascia. Dissection was made under the lateral thoracic fascia to release fascial flap from the thorax and abdominal wall (Fig. 4).

The silicone tube, hairless epithelial graft, and cartilage rings previously prepared were wrapped with lateral thoracic fascia on the most distal bifurcation of the lateral thoracic vessels. The skin incision was sutured with polypropylene 3/0 suture material.

In groups 3 and 4, tracheal cartilage was replaced with a Gore-Tex carotid vessel graft with a diameter of 6 mm. In the third group, the Gore-Tex graft was opened in the vertical plane. The hairless epithelial graft was adapted on pectoralis major muscle and then the inner surface of the Gore-Tex vessel graft was covered with this part of the muscle. The outer surface of the vessel graft was covered with the continuation of the bare muscle tissue. In this way, both surfaces of the Gore-Tex were surrounded by muscle. Prefabricated trachea formed by hairless epithelial graft, Gore-Tex, and muscle were wrapped around

Fig. 2 - *Ear cartilages were prepared in the form of tracheal cartilage rings.*

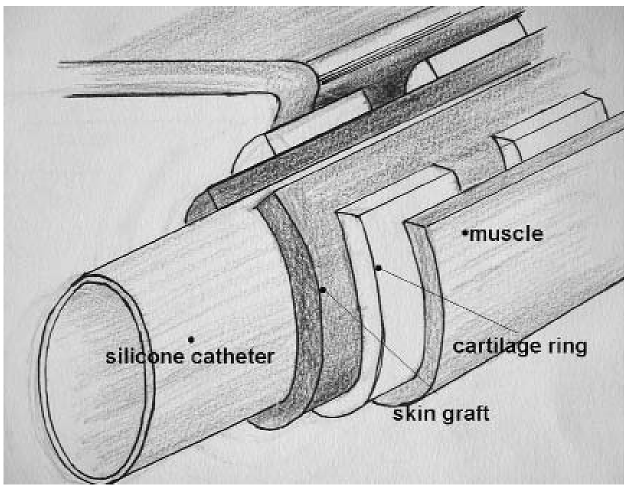

Fig. 3 - *Illustration depicting relation of muscle, cartilage rings and epithelial graft.*

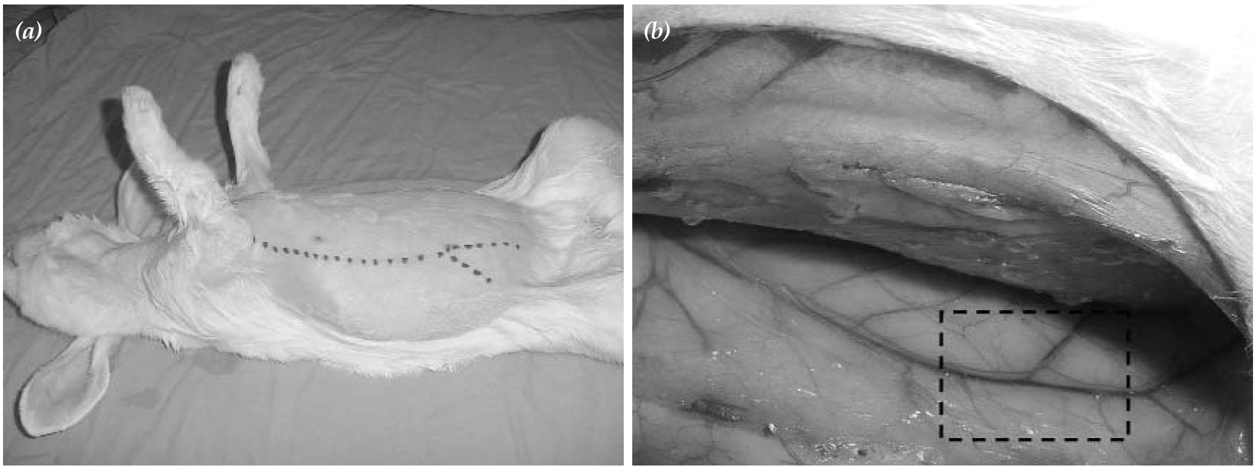

Fig. 4 - *Preparation of the lateral thoracic fascia.* ***(a)*** *Lateral thoracic vessels can be inspected externally.* ***(b)*** *Skin with underlying lateral thoracic fascia is dissected and released from the abdominal and thoracic wall. Dashed lines indicate the place where cartilage rings, hairless epithelial graft, and silicone tube will be adapted.*

the silicon catheter. In the fourth group, lateral thoracic fascia was used instead of pectoralis major muscle with similar surgical technique as in the third group (Fig. 5).

In groups 5 and 6, polypropylene mesh was used instead of tracheal cartilage. The polypropylene meshes having a rectangular shape (2x2 cm) were placed between the muscle or fascia and hairless epithelial graft (Fig. 6).

In groups 1, 3, and 5, the prefabricated trachea was left subcutaneously in the thoracal wall for future evaluation. In groups 2, 4, and 6, the prefabricated trachea was kept subcutaneously in the upper abdominal region for the same procedure to be applied.

Anastomosis to the native trachea was not performed; only the viability of tissue components, lateral flexibility, rigidity, wall thickness, and internal diameter of prefabricated neotrachea were evaluated.

The rabbits were evaluated macroscopically in the postoperative two weeks for wound healing. Intramuscular sulbactam ampicilline was applied daily in the first week after operation. The animals were fed with normal rabbit diet.

The experimental groups were compared using the ANOVA test with respect to viability, longitudinal flexibility, and graft necrosis of the axial flaps.

RESULTS

None of the animals had wound healing problems and all survived without infection or wound dehiscence. Two weeks later, silicon catheters were removed and prefabricated flaps were examined macroscopically for viability of hairless epithelial graft, longitudinal flexibility, rigidity, thickness of flap wall, internal diameter, and flap viability. Then, all prefabricated flaps were fixed in 5% glutaraldehyde solution, sectioned and stained with hematoxylin-eosin for histological examination.

The results of macroscopic examination are shown in Table II.

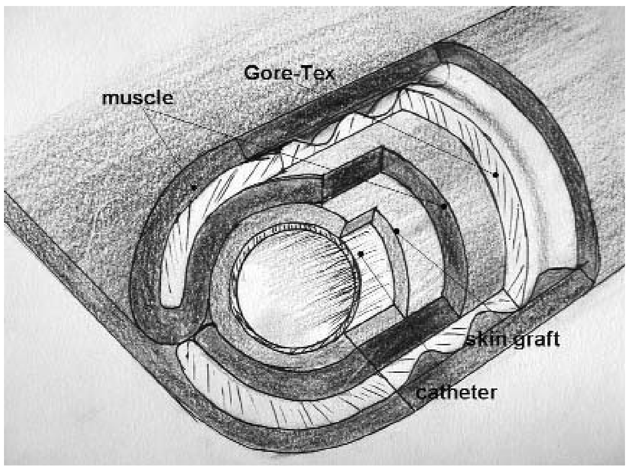

Fig. 5 - *Illustration of prefabrication in groups 3 (muscle) and 4 (fascia).*

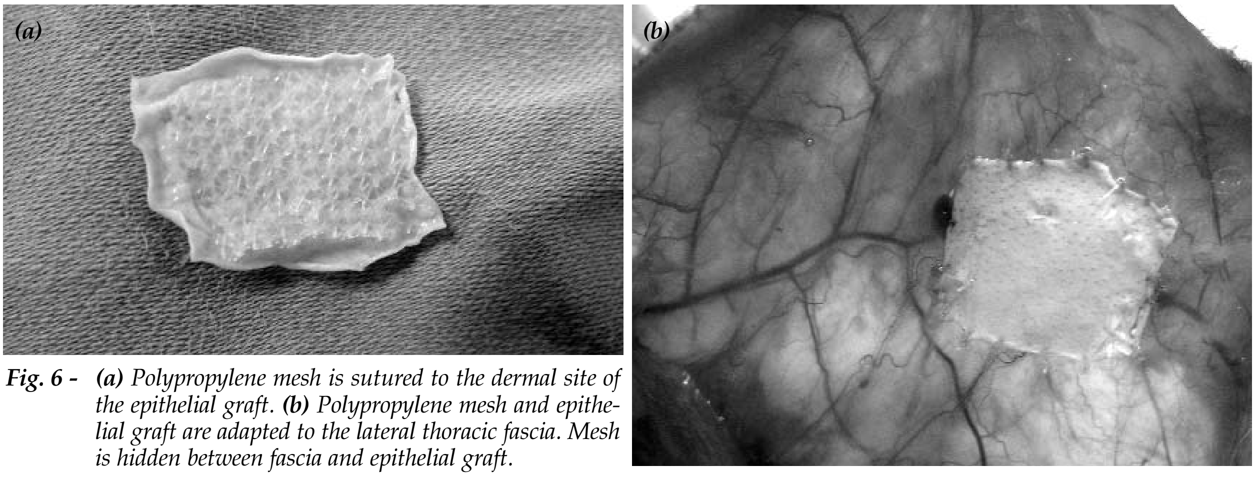

*Fig. 6 - **(a)** Polypropylene mesh is sutured to the dermal site of the epithelial graft. **(b)** Polypropylene mesh and epithelial graft are adapted to the lateral thoracic fascia. Mesh is hidden between fascia and epithelial graft.*

Viability of hairless epithelial graft: In groups 1 and 2, in which cartilage rings were used as a skeletal framework, 50% of the inner surface of the hairless epithelial graft was necrotic and edematous. Skin graft necrosis was more prominent in groups 3 and 4 in which Gore-Tex was used. No or little skin necrosis was observed in groups 5 and 6 in which polypropylene mesh was used.

Longitudinal flexibility: Longitudinal maximum angular flexibility was evaluated of all prefabricated flaps. Longitudinal flexibility was maximum in groups 1 and 2, in which cartilage rings were used, and was least in Gore-Tex groups. In groups 5 and 6 in which polypropylene mesh was used, longitudinal flexibility was similar to that of original trachea. Compared to the groups in which pectoralis major muscle was used for vascular supply, lateral thoracic fascia groups exhibited more flexibility.

Rigidity: After removing the silicon catheter, we observed that the prefabricated flaps showed more resistance to collapse in the Gore-Tex and polypropylene mesh groups, whereas groups in which cartilage rings were used showed the least resistance. The size of the cartilage rings did not change, but they became softer due to resorption, eventually causing collapse of the prefabricated trachea in these groups. The collapse was maximum in group 1 in which cartilage rings were used for skeletal support and pectoralis major muscle was used for vascular supply. In this group, just after removal of the silicon catheter, the muscle tissue herniated through gaps between the cartilage rings causing total obstruction of the lumen. A similar result was observed in group 2 in which fascia was used; however, this did not result in total obstruction of the lumen due to the thinner structure of the fascia.

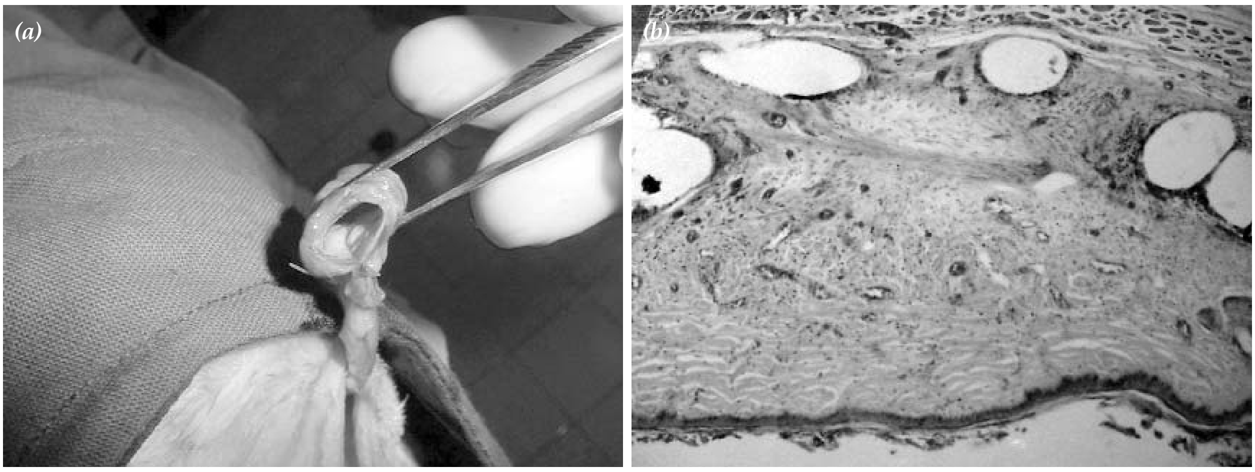

*Fig. 7 - **(a)** Macroscopic and **(b)** histological (H-E x 100) appearances of the axial bio-synthetic flap in group 6.*

TABLE II

THE RESULTS OF MACROSCOPIC EXAMINATION

		Necrosis of HEG (%)	Flexibility (°)	Collapse	Wall thickness (mm)	Internal diameter (mm)	Flap viability
Group 1	1	50	70	+++	3	0	+++
	2	55	75	+++	3	0	+++
	3	45	74	++	5	2	+++
	4	45	74	+++	4	0	+++
	5	50	76	+++	4	0	+++
	Mean	49	73.8				
Group 2	1	50	78	++	4	2	+++
	2	45	77	++	4	2	+++
	3	50	80	++	3	1	+++
	4	50	83	++	4	2	+++
	5	55	81	++	4	2	++
	Mean	50	79.8				
Group 3	1	75	40	–	5	4	+++
	2	70	42	–	6	5	+++
	3	80	40	–	5	5	++
	4	75	38	–	5	4	+++
	5	70	35	–	5	5	+++
	Mean	74	39				
Group 4	1	70	40	–	4	5	++
	2	70	40	–	3	5	+++
	3	75	43	–	3	5	++
	4	70	38	–	4	6	+++
	5	70	44	–	3	6	+++
	Mean	71	41				
Group 5	1	10	45	–	3	6	+++
	2	10	46	–	3	5	+++
	3	0	48	–	3	5	+++
	4	5	45	–	3	5	+++
	5	0	44	–	2	5	+++
	Mean	5	45.6				
Group 6	1	0	44	–	2	4	+++
	2	0	45	–	2	5	+++
	3	0	47	–	3	5	+++
	4	5	43	–	2	5	+++
	5	0	42	–	2	5	+++
	Mean	1	44.2				

HEG: Hairless epithelial graft; Collapse: No collaps (-), minimal (+), moderate (++), full (+++); Flap viability: moderate (++); good (+++).

Thickness of flap wall: In general, the thickness of the flap wall was greater in groups where muscle was used. The highest and lowest flap wall thicknesses were observed in group 3 (Gore-Tex and muscle) and group 6 (fascia and polypropylene mesh), respectively.

Internal diameter: Because of collapse, the luminal diameter was smaller in groups in which cartilage was used, particularly in group 1 where there was maximum collapse resulting in maximum luminal obliteration. The widest lumen was noted in group 3 and 4 in which Gore-Tex was used as a skeletal framework (Table II).

Flap viability: The least viability of flaps was observed in groups 3 and 4 in which Gore-Tex was used as a skeletal framework. The tissue lining the lumen was almost totally necrosed, but the outer part of the flap around Gore-Tex was viable. In the other groups, there was no problem of flap viability not only for luminal structure but also for the whole flap.

The results of histological examination showed good correlations with macroscopic findings (Fig. 7). Based on macroscopic and histological examinations, there was no statistically significant difference in viability of the flaps in all groups (p=0.694).

The ratio of skin graft necrosis differed significantly between the groups ($p<0.05$). There was no significant difference between group 1 and 2 with respect to skin graft necrosis ($p>0.05$). On the other hand, significant differences were noted in flexibility ($p<0.05$).

DISCUSSION

As Belsey[5] described, reconstructed trachea should have the following basic properties: lateral rigidity, vertical elasticity, airtight lumen, continuous internal lining of respiratory mucosa, and reliable healing.

None of the defined techniques in the present literature fulfill these criteria completely, especially if continuous internal lining of respiratory mucosa and lateral wall rigidity is concerned. Partial thickness and noncircumferential full-thickness tracheal defects have been reconstructed by autologous tissues successfully,[4,6,7] but reconstruction of wide circumferential tracheal defects is still a problem.[1,3,8,9]

Several methods and materials have been proposed for tracheal reconstruction, but with limited success. A wide variety of synthetic materials such as hydroxiapatite, Dacron, polyurethane, and polytetrafluoroethylene have been used, but all have resulted in disappointment.[10,11] Lack of blood circulation, inflammatory reactions, failure of anastomosis, infection, and probable exposition are the disadvantages of these synthetic reconstruction methods. Moreover, insufficient epithelial lining causes ingrowth of granulation tissue into the lumen resulting in occlusion of the lumen and stenosis. Autografts either single (cartilage, perichondrium, periosteum) or combined (cartilage/perichondrium, cartilage/mucosa) have also been tried.[4,12,13]

Axial flaps, due to rich blood supply, cause minimal inflammation and provide optimal circulation for anastomosis healing compared to synthetic and autologous grafts. Many axial flaps such as sternocleidomastoid muscle with clavicular periosteum[14] or pectoralis major[15] muscle have been proposed for tracheal reconstruction. However, these methods both have the disadvantages of lack of skeleton support and epithelial lining. To solve these problems, intercostal muscle flap[16] raised with cartilage and combined with hairless epithelial graft has been proposed. This technique also did not get much of a chance to be used due to high rate of complications and mortality.

Flap prefabrication studies for tracheal reconstruction were started in 1990's. Before then, free jejunal transfers were used, but not with satisfactory results. Costantino et al.[17] used Dacron mesh prefabricated jejunal flaps. Cavadas[3] used autologous tissues for tracheal prefabrication in goats with partial success. Delaere et al.[4] who used lateral thoracic fascia as a vascular carrier in prefabrication examined all the combinations of mucosa, cartilage graft and fascia. Ruuskanen et al.[18] prefabricated perichondrial flaps to form cartilaginous tubes. Fayad and Kuriloff[19] prefabricated well-vascularized axial muscular flap tubes supported by collagen and morphogenetic protein. All these flaps provided optimal circulation and rigidity, but lacked epithelial lining. Cavadas[3] prefabricated an axial composite flap consisting of skin cartilage and mucosa which served as epithelial lining, but the method was complicated and required multiple procedures.

In this study, we tried to find the most appropriate autologous tissue and alloplastic material combination to substitute basic properties of the native trachea.

In groups 3 and 4 (Gore-Tex), we observed the least viability of the hairless epithelial graft because the compression of the urinary catheter applied as a stent for hairless epithelial graft caused necrosis of the luminal structures due to the inelasticity of the surrounding Gore-Tex. Also the kinks in the edges of the Gore-Tex decreased circulation to luminal structures. In groups 5 and 6 (polypropylene mesh), the viability of the hairless epithelial graft was excellent because porous structure of the polypropylene mesh allowed graft feeding from vascular supply. In groups 1 and 2 (cartilage rings), the hairless epithelial graft could not take enough circulation between the cartilage rings which were partially resorbed and inflamed, causing graft necrosis.

The longitudinal flexibility was similar to that of original trachea in groups 5 and 6 (polypropylene mesh). In groups 1 and 2, due to partial resorption of cartilage rings, there was increased longitudinal flexibility. The cartilage rings were not fixed to each other and were moving separately. The longitudinal flexibility of the Gore-Tex groups (group 3 and 4) was slightly less than that of polypropylene mesh groups probably because of relatively increased wall thickness.

Luminal collapse was mostly seen in groups 1 and 2; the reason seems to be the herniated muscle tissue and fascia between the cartilage rings and loss of rigidity in skeletal structure due to partial resorption of cartilage rings. Also muscle contraction may have contributed to herniation in group 1. Strong fibrovascular connections between vascular supply and hairless epithelial graft through network of polypropylene mesh prevented collapse in groups 5 and 6. In Gore-Tex groups, there was no collapse in skeletal structure because of excellent rigidity of Gore-Tex; however, necrosis was seen in luminal structures and there was collapse of the necrotic luminal structures (hairless epithelial graft and vascular supply) because of lack of attachment between Gore-Tex and luminal structures in these groups. We presume that, even in the presence of viable luminal structures, there would again be luminal collapse due to lack of attachment between Gore-Tex and luminal structures.

Lateral thoracic fascia has many advantages over pectoralis major muscle in terms of vascular supply; it is thinner and easily manipulated and its longer pedicle can be used in any kind of prefabrication. Contraction could be a problem for pectoralis major in the long-term and it leaves functional and aesthetic deformity, as well.

The wall thickness and internal diameter were similar to original trachea in polypropylene mesh groups, showing that polypropylene mesh served as an excellent substitute for skeletal framework and allowed inner lining viability while maintaining a thin rigid wall. In Gore-Tex groups, internal diameters were wider and this was probably due to necrosis seen in the inner parts of the prefabricated flap.

In a previous study reporting the use of an axial biosynthetic flap, we achieved an ideal tracheal reconstruction by transferring a polypropylene mesh prefabricated in lateral thoracic fascia.[20] With this preliminary study, we aimed to compare different prefabricated models without anastomosis to the native trachea. Our results suggest that polypropylene mesh used with lateral thoracic fascia as a vascular supply seems to be the best biosynthetic combination in the reconstruction of circumferential tracheal defects.

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