Available online at www.sciencedirect.com
SCIENCE DIRECT®

Thermochimica Acta 445 (2006) 190–194

thermochimica acta

www.elsevier.com/locate/tca

Differential scanning calorimetric examination of the tracheal cartilage after primary reconstruction with differential suturing techniques

Zalán Szántó [a,*], Gyula Kovács [a], Viktor Nagy [a], Elizabeth Rőth [b], Thomas F. Molnár [a], Örs P. Horváth [a]

[a] *Surgical Clinic, Department of Thoracic Surgery, University of Pécs, Faculty of Medicine, H-7624 Pecs, Ifjusag Str. 13, Hungary*
[b] *Department of Surgical Research and Techniques, University of Pécs, H-7624 Pecs, Ifjuság Str. 13, Hungary*

Available online 13 October 2005

Abstract

Objective: Long segmental tracheal defects often lead to life threatening clinical conditions. Treatment of these lesions still represents an unsolved surgical challenge. The aim of the study was to test the effect of continuous and simple interrupted suturing in the replacement of long segment tracheal defects using polytetrafluoroethylene (PTFE) vascular prosthesis in a rabbit model.
Methods: 2 cm long segment of the cervical trachea was resected in 20 New Zealand rabbits. The trachea was replaced with reinforced polytetrafluoroethylene vascular graft. The anastomoses were performed telescopically using continuous (group I, $n = 10$) or simple interrupted sutures (group II, $n = 10$). Laser Doppler measurements were taken before the resection and following the anastomoses. Length of survivals were noted, the patency and microscopical pattern of the anastomoses were evaluated. Calorimetric examinations were performed to detect possible structural changes in the tracheal cartilage.
Results: Following the resection local microcirculation decreased by $9 \pm 4\%$. The anastomoses caused a significant decrease of 29% in group I ($p = 0.02$) and 13% in group II. The mean survival was 58 ± 17 and 135 ± 25 days, respectively. Calorimetric results showed no change after the resection, but significant shift in melting temperature and calorimetric enthalpy proved the presence of structural changes of the cartilage in group II.
Conclusions: We saw significant lowering of microcirculation following continuous sutures, while simple interrupted stitches produced only moderate decrease. We found that interrupted suture technique is superior to the continuous technique causing only moderate damage to the tracheal anastomosis.

Keywords: Trachea; Anastomosis; Suturing technique

1. Introduction

Long segmental tracheal defects are quite rare, but they often represent life threatening clinical conditions. Reconstruction of such lesions is still a surgical challenge and individually based [1]. The earliest reports of long segmental tracheal reconstructions were published in the middle of the last century. The authors investigated resection and replacement of the damaged trachea with autologous tissue or prosthesis [2,3], but poor outcome of the experiments hindered eventual human application. Since than several experiments have been performed to find a suitable artificial [4,5], or composite graft and technique for tracheal replacement, but results are conflicting [6,7]. Polytetrafluoroethylene (PTFE) grafts are used in many fields of surgery because of their superior tissue compatibility and flexibility. Trojan et al. [8] reported about positive results if using in tracheal replacement.

There are different techniques for tracheal end-to-end anastomosis, with the modification of the tracheal resection technique, suturing type and the applied suture material. Controversial reports highlight the fact that the suturing technique in the anastomosis is still subject to debate. Single interrupted sutures allow precise approximation of the tracheal ends, but continuous suturing gives more tensile strength to the anastomosis. To our knowledge investigators dealing with this very problem have only indirect evidence at hand based on post-operative results.

* Corresponding author. Tel.: +36 72 536 126; fax: +36 72 536 127.
E-mail address: szantozalan@yahoo.com (Z. Szántó).

0040-6031/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2005.09.003

We aimed to show the intra-operative effect of continuous and simple interrupted suturing technique on the tracheal microcirculation as well as the structural changes of the tracheal cartilage using differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Animal preparation and anaesthesia

All experiments were in accordance to rules and regulations of the European Convention on Animal Care regarding the use of animals in medical research. The study was approved by the local authorities of animal research (BA02/2000-16/2001). Thirty healthy adult New Zealand rabbits (15♀, 15♂, 2.7–4.5 kg) were randomly selected and denied access to food 6 h prior to surgery. The animals were sedated with xylazine hydrochloride (5 mg/kg) and ketamine hydrochloride (35 mg/kg) in addition to lidocaine hydrochloride injected at the operative site pre- and post-operatively.

2.2. Surgical procedure

Following a midline neck incision the neck muscles were separated to circumferentially expose the trachea below the larynx. We resected a 2 cm long segment of the cervical trachea with surgical blade. Haemostasis was achieved with bipolar coagulation and the use of haemostatic sponge (Surgicel, Johnson & Johnson, New Brunswick, NJ). After completing the resection, ventilation was maintained with a sterile tracheal tube through the distal stub. Reinforced PTFE (Gore Tex, W.L. Gore and Associates, Inc., Ø: 5 mm, length: 2 cm) was then grafted between the two tracheal stumps. We performed telescopic end-to-end anastomosis with continuous sutures ($n = 10$) or with interrupted sutures ($n = 10$) (PDS, 5/0, Ethicon, Inc., Somerville, NJ). Sutures were tension-free and traversed the tracheal ends in full thickness, incorporating one tracheal ring at each end. After completing the anastomosis operative area was closed according the appropriate surgical rules. The protocol included 180 days of follow-up period, but animals were prematurely euthanized if severe respiratory distress developed.

2.3. Laser Doppler measurements

Intra-operatively Laser Doppler measurements (MBFD3, Moor Instruments, England) were performed anteriorly in six different points on the proximal and distal resection lines. Triple measurements were taken before and after the resection and following completion of the anastomoses. An adjustable metal platform was designed to maintain a stable position of the Laser Doppler probe above the operative field.

2.4. Stenosis classification

The degree of airway narrowing was expressed as a percentage of the area of intact tracheal lumen using computer planimetry (NIH Image, RSB). Loss of patency was classed as less than 25, 25–50, 50–75%, and more than 75%. Although both proximal and distal anastomoses were examined in every case, each animal was grouped according to its more severe stenosis.

2.5. Sample preparation

After the follow-up period cartilage rings of the anastomotic area and an intact tracheal ring, which served as a control, were removed, and carefully derived from tissue fragments taken during the operation and considered to be waste material. Samples were washed three times in sterile phosphate-buffered saline (PBS, pH 7.4) in order to eliminate all extracartilaginal tissue remnants. Samples were then put into RPMI-1640 solution (SIGMA) containing 10% fetal bovine serum (HYCLONE laboratories), antibiotic, antimycotic solution (1 U/ml penicilline, streptomycine, gentamycine and fungisone, GIBCO Lab.), non-essential amino acids (GIBCO) and sodium carbonate. All the individual samples were stored separately at 4 °C, no longer than 24 h. Then samples were subjected to calorimetric measurement.

2.6. Differential scanning calorimetry

The thermal unfolding of healthy and operated trachea cartilage was monitored by a SETARAM Micro DSC-II calorimeter. All experiments were carried out between 0 and 100 °C with a scanning rate of 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with an average 850 μL sample volume. RPMI-1640 buffer was used as a reference sample. The sample and reference vessels were equilibrated with a precision of 0.1 mg. The SETARAM two points fitting integrating software calculated the calorimetric enthalpy.

2.7. Histological examination

On the histological work-up we investigated the tracheal cartilage of the resected segment and following the follow-up the graft along with both anastomotic stumps were harvested and preserved in 10% formaldehyde. Haematoxylin–eosin stained specimens of both ends and sections of the graft were examined using light microscopy, documenting the degree of granulation tissue and regeneration of tracheal epithelium.

2.8. Statistical analysis

Results are expressed as mean values ± S.E.M. Data were analyzed with one-way analysis of variance (ANOVA). The level of significance was set at $p < 0.05$. The Micro Cal Origin (Version 6.0) program (Microcal Software Inc., Northampton, USA) was used for graphical presentation.

3. Results

3.1. Microcirculation

Fig. 1 demonstrates alterations in microcirculatory values. Following the resection local microcirculation decreased by 11 ± 5% ($p = 0.18$). The anastomoses caused a significant

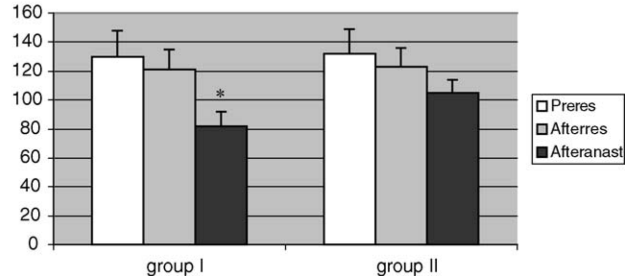

Fig. 1. The microcirculatory data before (empty column) and following (gray column) the tracheal resection and after completing the anastomosis (black column) in the two groups. Significant decrease was seen in group I, completed with continuous sutures ($^{*}p < 0.05$).

decrease of 29% in group I ($p = 0.02$) and 13% in group II ($p = 0.06$).

3.2. Survival

The mean survival was 55 ± 15 days in group I and 142 ± 27 days in group II. Various degrees of granulation tissue leading to different extents of airway narrowing are shown on Table 1.

3.3. Microscopical changes

There were no detectable differences between the resected cartilage compared to the cartilage samples involved in the anastomosis or control. The extracellular matrix showed its normal appearance, in some cases a slight chondrocellular oedema developed (group I, $n = 6$; group II, $n = 4$; Fig. 2). We examined the process of neoepithelisation along the upper and lower anastomotic lines and midway along the grafts. Signs of this process were seen in 22 cases on the inner surface of the grafts by animals with longer survival than 4–6 weeks. Complete neoepithelisation was seen in only six animals with pseudorespiratory epithelium yet lacking cilia (Fig. 3).

Table 1
Extend of tracheal stenosis in the groups

Group	Mean survival (days)	>75%	50–75%	25–50%	<25%
I	58 ± 17	1	2	4	3
II	135 ± 25	–	2	3	5

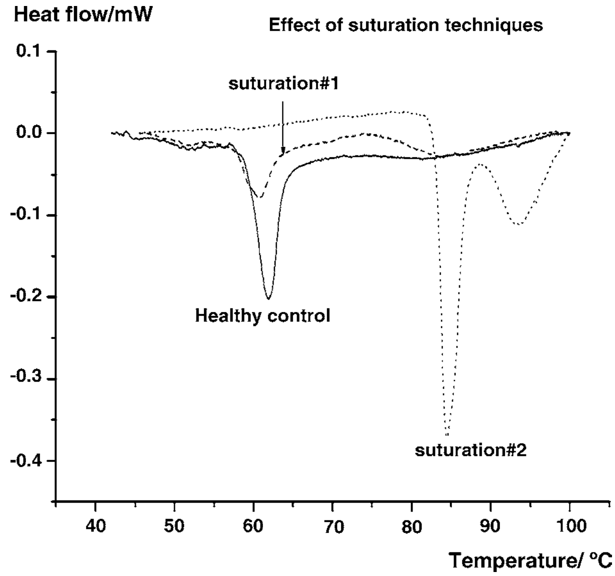

Fig. 3. DSC scans of the cartilage samples. Curves of the healthy (solid line) and the operated trachea (group I dotted, group II dashed) showed remarkable difference during the measurements.

3.4. DSC measurements

DSC scans of healthy and operated tracheal cartilages showed complex thermal denaturation characteristics (Fig. 4). We have

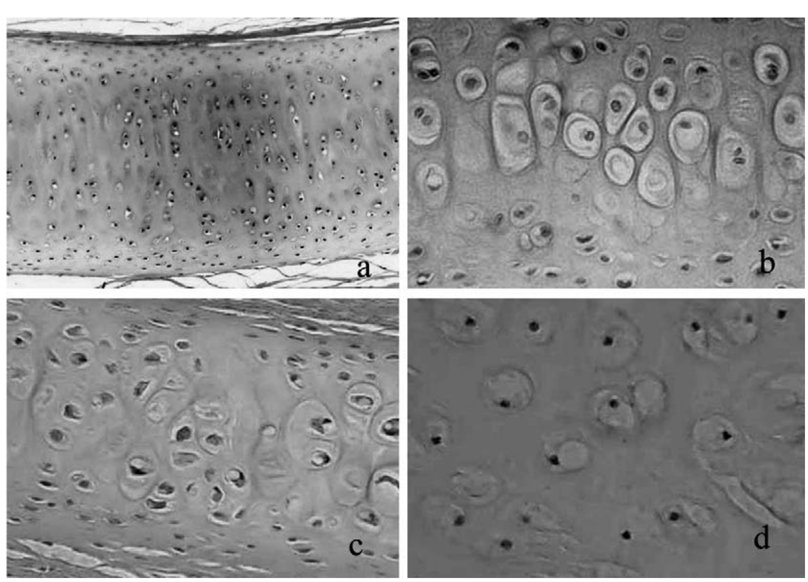

Fig. 2. Ultrastructural picture of the tracheal specimens with convential histological examination. There were normal structure in the resected and control cartilage (a, b) and were no detectable marked difference in cartilage cells and matrix after the anastomosis completed with continuous (group I, c) or interrupted sutures (group II, d).

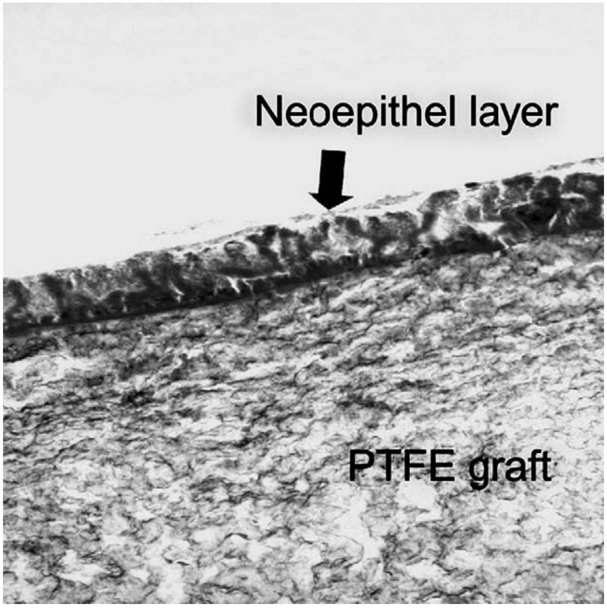

Fig. 4. Hystology of the PTFE graft with neoepithelization of the inner surface (group II, survival 180 days).

focused our attention to the components of high melting temperature. The proper thermal parameters of the main transition in average were $T_m = 61.8\,^\circ C$ and $\Delta H = 0.49$ J/g for healthy and $T_m = 60.6\,^\circ C$ as well as $\Delta H = 0.55$ J/g were obtained for samples operated with interrupted sutures. The effect of continuous sutures is manifested in the increased T_m, of the marked second endoterm peak at 83 °C that increased the transition enthalpy by 10% too. The influence of the continuous suturation was more pronounced on the first cooling curve (not shown). The high temperature melting seems to be reversible for healthy sample in 5% while disappears for both operation techniques. The low temperature transition during renaturation was observed at the same temperature with the same calorimetric enthalpy for healthy and interruptedly sutured sample and appeared during the second reheating too in same manner at around 25 °C with the same enthalpy change. Tracheal rings with continous sutures showed significantly different high melting characteristics for the first heating cycle. The denaturation temperatures were shifted to 83.9 and 96.3 °C with a total calorimetric enthalpy of 0.335 J/g. The denaturation was totally irreversible, no heat effect could be observed during the first cooling and second reheating process.

4. Discussion

Primary reconstruction after segmental tracheal resection is possible following the extended lesion up to one half of the trachea in adults and one third in infants and small children [1,9]. By lesions extending this length surgical treatment represents an unsolved problem. Several investigators have been trying to find the ideal method and tracheal graft for replacement but the results are controversial. McKeown et al. [10] compared the healing of primary tracheal anastomosis of infant New Zealand rabbits completed with continuous or interrupted sutures. They found significantly less stenosis when an absorbable suture material and interrupted sutures were employed. Fingland et al. [11] compared single continuous sutures versus simple interrupted sutures after extensive tracheal resection in a dog model. They reported that completing the anastomosis with continuous sutures led to a less precise apposition and significantly greater stenosis. Behrend et al. [12] performed a resection of four tracheal rings and completed primer anastomosis with interrupted or continuous suturing in a sheep model. They did not find principal difference of healing between the two techniques.

To investigate the acute, intra-operative effects of suture techniques we monitored tracheal microcirculation using Laser Doppler. Animals in group I anastomosed with continuous sutures had significant reduction of the local microcirculation. In group II where interrupted sutures were employed this decline was not observed. According to the results simple interrupted sutures cause less strangulation of the supplying arteries of the trachea than continuous sutures.

In the present study we used reinforced PTFE grafts for long segment defects in a rabbit model. PTFE grafts, mainly used in vascular surgery are widely known to have reliable tissue compatibility although its application in tracheal surgery is reported, the results have been inconclusive [8,13]. Using reinforced PTFE grafts we were able to replace long segmental tracheal defects without experiencing any acute post-operative complications.

In our study graft neoepithelisation was seen in the majority of the animals, but was complete only in six cases. We observed the start of this process in animals whose survival was longer than 30–40 days independently of the anastomotic technique and suture type. It seems that the anastomotic technique has no effect on the process of neoepithelisation, as it always appears in cases when the survival exceeds more than 4–6 weeks.

Granulation tissue formation is still a major concern in tracheal surgery, starting soon after reconstruction and causing severe breathing difficulties. In our study groups completed with continuous sutures had relatively low degree of stenosis, due to shorter animal survival determining tissue in-growth progression. When interrupted sutures were used, airway shrinkage was more advanced, as longer survival allowed progression of this process.

Tracheal rings are built up of hyaline cartilage, which forms a supporting framework to resist the pressure changes in the airways. It is composed of chondrocytae, cartilage matrix (collagen type II, IX, X, XI), proteins of of non-collagen type (proteoglycans), inorganic materials and water. Collagen fibres are responsible for the tensile strength of the tracheal cartilage, while proteoglycans are responsible for compressibility.

Effect of ischaemia on the tracheal cartilage is not perfectly understood, as conventional histological examination cannot show any marked difference of chronic cartilage damage. Differential scanning calorimetry can detect structural characteristics of different biological samples by analysing heat flow by the elevation of the temperature [14–19]. Than et al. [20,21] demonstrated that DSC is a useful method for the investigation of hyaline cartilage in different stages of human as well as rabbit osteoarthritis [22]. Doman et al. [23,24] presented similar data on healthy and degenerated nucleus pulposus and anulus fibrosus of human invertebral disc. They explained the pronounced heat capacity change between intact and arthritic samples with the structural alterations in osteoarthritis caused by ischaemic biochemical processes [25].

With our investigations we could demonstrate that DSC is a useful and well applicable method for the investigation of tracheal cartilage. The changes in thermal conditions of the cartilage are evoked by the surgical intervention proved the structural shift of the operated cartilage. The thermal behaviour of the operated area showed a stiffer structure than the non-operated field, which may be the result of the anastomotic sutures and the decrease of the local circulation. Differences between the DSC curves of the groups operated with continuous and interrupted technique indicate the particular effects of the two suturing techniques. We saw more severe structural changes after continuous sutures causing the lack of healing.

Complications after long segmental tracheal replacement such as tracheal necrosis can be positively influenced using interrupted suturing, as this only moderately decreases the local microcirculation at the tracheal stumps and causes less structural damage of the tracheal cartilage than continuous sutures.

Acknowledgements

This work was supported by grants of OTKA T035790, NFKP/1/A00026/2002.

References

[1] H.C. Grillo, Ann. Thorac. Surg. 73 (6) (2002) 1995.
[2] R. Belsey, Br. J. Surg. 38 (1950) 200.
[3] D.W.J.J. Ferguson, O.H. Wangensteen, Surgery 28 (1950) 597.
[4] R.R. Shaw, A. Aslami, W.R. Webb, Ann. Thorac. Surg. 5 (1) (1968) 30.
[5] O.V.-J.J. Villegas-Cabello, F.M. Gutierrez-Perez, R.F. Davila-Cordova, C. Diaz-Montemayor, Thorac. Cardiovasc. Surg. 42 (1994) 302.
[6] P.D. Costantino, D.W. Nuss, C.H. Snyderman, J.T. Johnson, C.D. Friedman, K. Narayanan, G. Houston, Ann. Otol. Rhinol. Laryngol. 101 (10) (1992) 807.
[7] M. Hirano, T. Yoshida, S. Sakaguchi, Ann. Otol. Rhinol. Laryngol. 98 (9) (1989) 713.
[8] I. Trojan, L. Kecskes, B. Vecsei, S. Bense, M. Brzozka, B. Ordogh, Z. Farkas, Thorac. Cardiovasc. Surg. 33 (6) (1985) 337.
[9] H.C. Grillo, Ann. Thorac. Surg. 49 (6) (1990) 864.
[10] P.P. McKeown, H. Tsuboi, T. Togo, R. Thomas, R. Tuck, D. Gordon, Ann. Thorac. Surg. 51 (4) (1991) 636.
[11] R.B. Fingland, C.I. Layton, G.A. Kennedy, J.C. Galland, Vet. Surg. 24 (4) (1995) 320.
[12] M. Behrend, J. Klempnauer, Eur. Surg. Res. 33 (3) (2001) 210.
[13] D.L. Cull, K.P. Lally, E.A. Mair, M. Daidone, D.S. Parsons, Ann. Thorac. Surg. 50 (6) (1990) 899.
[14] D. Lőrinczy, F. Könczöl, B. Gaszner, J. Belagyi, Thermochim. Acta 322 (2) (1998) 95.
[15] D. Lőrinczy, J. Belagyi, Thermochim. Acta 259 (1) (1995) 153.
[16] D. Lőrinczy, J. Belagyi, Biochem. Biophys. Res. Commun. 217 (2) (1995) 592.
[17] D. Lőrinczy, J. Belagyi, Thermochim. Acta 296 (1–2) (1997) 161.
[18] D. Lőrinczy, J. Belagyi, Eur. J. Biochem. 268 (22) (2001) 5970.
[19] D. Lőrinczy, N. Hartvig, J. Belagyi, J. Biochem. Biophys. Methods 53 (1–3) (2002) 75.
[20] P. Than, I. Domán, D. Lőrinczy, Thermochim. Acta 415 (1–2) (2004) 83.
[21] P. Than, Cs. Vermes, B. Schäffer, D. Lőrinczy, Thermochim. Acta 346 (1–2) (2000) 147.
[22] P. Than, D. Lőrinczy, Thermochim. Acta 404 (1–2) (2003) 149.
[23] I. Domán, T. Illés, D. Lőrinczy, Thermochim. Acta 405 (2) (2003) 293.
[24] I. Domán, G. Tóth, T. Illés, D. Lőrinczy, Thermochim. Acta 376 (2) (2001) 117.
[25] T.F. Molnar, J. Hasse, K. Jeyasingham, M.S. Rendeki, Ann. Thorac. Surg. 77 (1) (2004) 372.