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DIFFERENTIAL SCANNING CALORIMETRIC EXAMINATION OF THE TRACHEAL CARTILAGE AFTER PRIMARY RECONSTRUCTION WITH DIFFERENTIAL SUTURING TECHNIQUES

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Resection and subsequent end-to-end anastomosis of the windpipe is a tried-and-tested acceptable method for the surgical treatment of segmental defects. There are a variety of different techniques for tracheal end-to-end anastomosis, but controversial reports highlight the fact that the suturing technique of the anastomosis is still subject of debate. We aimed to show the postoperative effects of the continuous and simple interrupted suturing technique respectively on the tracheal cartilage using differential scanning calorimetry. Transsection and subsequent reanastomosis of the cervical trachea was performed in 14 adult beagle dogs. The trachea was anastomized with continuous or simple interrupted sutures respectively depict no change in microcirculation after the resection of the trachea, but significant decrease following the completion the anastomosis with continuous sutures. Conventional histological analysis did not show any marked postoperative change in the tracheal cartilage but our DSC scans clearly demonstrated the differences between the intact cartilages and the ones involved in the anastomosis.

Keywords: *continuous and interrupted sutures, DSC, end-to-end anastomosis, tracheal cartilage*

Introduction

Acquired upper airway stenosis is usually associated with a complex of pathological conditions at the high tracheal and the subglottic levels. Reported reconstructive techniques include widening by incorporation of grafts, segmental resection, and anastomosis or combined procedures. Progress in anaesthesia, surgical techniques, and understanding of the pathophysiology of the trachea has made tracheal reconstruction a safe operative procedure, although there are no reports observing its acute and short term effect on the tracheal cartilage.

There are a variety of different techniques for tracheal end-to-end anastomosis, including the modification of the tracheal resection technique, suturing type and the applied suture material [1–5]. Controversial reports highlight the fact that the suturing technique in the anastomosis is still subject of debate. Continuous suturing technique seems to offer an easy method for the reconciliation of the tracheal stumps, as it provides an even distribution of tension around the tracheal lumen [2]. Other authors emphasize the beneficial post-operative effect of the interrupted suturing technique [5]. To our knowledge investigators dealing with this very problem have only indirect evidence at hand that is based on postoperative results. We aimed to detect the possible changes of the tracheal cartilage after primary tracheal reconstruction with two different suturing techniques. We used DSC technique because we have a lot of experience in the field of thermal denaturation of biological macromolecules [6–13] to follow the thermal consequences of any artificial intervention in their structure.

Hypothesis-objectives

Our hypothesis was that after resection of the windpipe and completing the end-to-end anastomosis there is a decrease in the tracheal microcirculation resulting in deformation of the tissue elements building up the cartilage. We examined the healthy cartilage in contrast to the cartilage involved in the tracheal anastomosis.

Our aim was to prove with the examinations that there is a definitive difference in the structure of the healthy cartilage and that of involved in the anastomosis.

Objectives of research were:

- Introduction of the application of a new method in tracheal research.
- Setting up of calorimetric standards of normal tracheal cartilage.
- Applying calorimetric methods for the investigation of cartilage involved in the anastomosis. Pre-

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sentation of the differences in the samples of normal and changed conditions.
- Comparing between two suturing techniques in tracheal primary reconstruction.

Experimental

Materials and methods

Animal preparation and anaesthesia

All experiments were in accordance to rules and regulations regarding the use of animals in medical research. The study was approved by the local authorities (BA02/2000-16/2001). 14 healthy adult beagle dogs (4 female, 3 male, 12–19 kg) were randomly selected and denied access to food 6 h prior to surgery. Premedication was performed with Droperidol (1.5 mg kg^{-1}), Fentanyl (0.03 mg kg^{-1}) and Atropin (1 mg). Anaesthesia was induced with i.v. Thiopental-sodium (5–10 mg kg^{-1}) and maintained with 0.5% halothane in 70% nitrous oxide and 30% oxygen gas mixture. Lidocaine hydrochloride was injected at the operative site pre- and postoperatively.

Surgical procedure

Following a midline neck incision the neck muscles were separated to circumferentially expose the trachea below the larynx. Circumferential tracheal transsection was performed with a surgical blade between the 3rd and 4th tracheal rings distally to the cricoid cartilage. Haemostasis was achieved with bipolar coagulation and the use of haemostatic sponge (Surgicel, Johnson & Johnson, New Brunswick, NJ). After completing the transsection, ventilation was maintained with a sterile tracheal tube through the distal airways. We performed primary end-to-end reanastomosis with continuous sutures (n=7) or with interrupted sutures (n=7) (PDS, 3/0, 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 the operative site was closed according the appropriate surgical rules.

The animals were sedated and painlessly euthanized after 20 days of follow up.

Laser Doppler measurements

Laser Doppler measurements (MBFD3, Moor Instruments England) were performed anteriorly at three different points on the first proximal and distal tracheal rings related to the resection lines, and on the 8th tracheal ring, which served as control in the experiments. 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.

Histological analysis

After sacrificing the animal, the anastomotic stumps were harvested and preserved in 10% formaldehyde. Haematoxylin-eosin stained specimens were examined using light microscopy, documenting the postoperative changes of the tracheal cartilage.

Sample preparation

After the follow up period two rings of the anastomotic area and the control were sampled, and carefully derived from tissue fragments. All the cartilage samples weighed identically ca. 100 mg, 5 mm in length, 5 mm in width and had a height of 3 mm. Samples were washed three times in PBS (sterile phosphate-buffered saline, 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^{-1} 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.

DSC measurements

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^{-1}. 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. It was not necessary to correct for heat capacity between the sample and reference vessels. The Setaram two points fitting integrating software calculated the calorimetric enthalpy.

Statistical analysis

Results are expressed as mean values ±SEM. Data were analysed with 1-way analysis of variance (ANOVA). The level of significance was set at $P<0.05$. The Micro Cal Origin (ver. 6.0) program (Microcal Software Inc. Northampton, USA) was used for graphical presentation.

Results and discussion

McKeown *et al*. compared the healing of primary tracheal anastomosis of infant New Zealand rabbits completed with continuous or interrupted sutures [14]. They found significantly less stenosis when an absorbable suture material and interrupted sutures were employed. Ndiaye [15] has achieved good results during the growth of tracheal sutures with absorbable sutures in primates. Fingland *et al*. compared single continuous sutures *vs*. simple interrupted sutures after extensive tracheal resection in a dog model [16]. They reported that completing the anastomosis with continuous sutures led to a less precise apposition and significantly greater stenosis. Behrend *et al*. [4] performed a resection of 4 tracheal rings and completed primary anastomosis with interrupted or continuous suturing in a sheep model. They did not find principal difference of healing between the two techniques.

Microcirculation

Figure 1 demonstrates alterations in microcirculatory values. Results depict no change after the resection of the trachea, but significant decrease following the completion the anastomosis with continuous sutures. With the use of interrupted sutures significant decrease was not present. No effect was seen on the control tracheal rings.

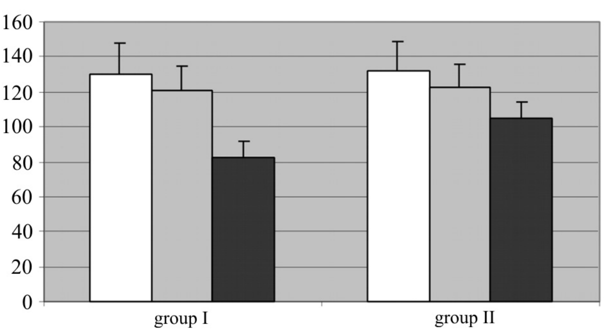

Fig. 1 The microcirculatory data before (empty column) and following (gray column) the tracheal resection and after completing the anastomosis (black column) in the two groups. Significant decrease was seen in group I, completed with continuous sutures (*$p<0.05$)

Histology

Figure 2 shows the light microscopical pictures of the tracheal specimens. The 8th tracheal ring was without any difference after surgery, but there were no impressive change in the cartilage specimens involved in the anastomosis. With this method we could not detect any difference between the two different operative techniques.

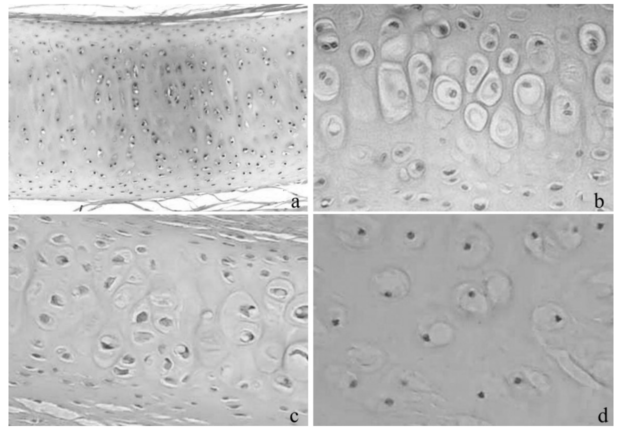

Fig. 2 Ultrastructural picture of the tracheal specimens with conventional histological examination. There was normal structure in the control cartilage (a, b). There were no detectable marked difference in cartilage cells and matrix after the anastomosis completed with continuous (group I, c) or interrupted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

DSC measurements

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 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 [17].

Effect of ischaemia on the tracheal cartilage is not perfectly understood, as conventional histological examination cannot show any marked difference of acute cartilage damage. Than *et al*. demonstrated that DSC is a useful method for the investigation of hyaline cartilage in different stages of human [18, 19] as well as rabbit osteoarthritis [20]. Doman *et al*. presented similar data on healthy and degenerated nucleus pulposus and anulus fibrosus of human invertebral disc [21, 22]. They explained the pronounced heat capacity change between intact and arthritic samples with the structural alterations in osteoarthritis caused by the biochemical processes.

DSC scans of healthy and operated tracheal cartilages showed complex thermal denaturation characteristics (Fig. 3). We have 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°C and ΔH=0.49 J g^{-1} for healthy and T_m=60.6°C as well as ΔH=0.55 J g^{-1} were obtained for samples operated with continuous sutures. The effect of continuous sutures is manifested in the increased T_m of the marked second endotherm 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 temper-

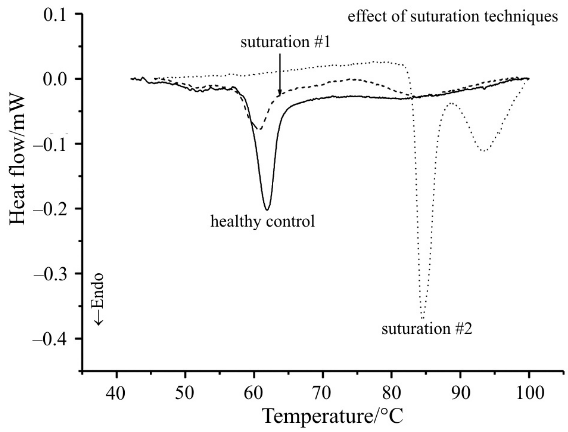

Fig. 3 DSC scans of the cartilage samples, curves of the healthy (solid line) and the operated trachea (group #1 dashed, group #2 dotted) showed remarkable difference during the measurements

ature melting seems to be reversible for healthy sample in 5% while perfectly 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 continuously suturated sample and appeared during the second reheating too in same manner at around 25°C with the same enthalpy change. Tracheal rings taken from dogs with interrupted suturation techniques showed a 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^{-1}. The denaturation was totally irreversible: no any heat effect could be observed during the first cooling and second reheating.

With our investigations we could demonstrate that DSC is a useful and well applicable method for the investigation of tracheal cartilage. The structural changes evoked by surgical intervention could be monitored too. There were marked difference between the DSC curves of the groups operated with continuous and interrupted technique.

We believe that numerous open questions have to be cleared in the future:

- Which components of the cartilage are mainly responsible for the demonstrated DSC findings? In order to answer it additional separation of different components with biochemical methods is necessary as well as the establishment of DSC features of each component.
- What is the effect of varying influencing factors of microcirculation in detail? Can the different surgical techniques of trachea be demonstrated on the DSC scans as well? Are the thermal curves of dogs differing from each other by age, gender significantly dissimilar?
- There is need for more sensitive histological techniques to follow the effect of ischaemia on the tracheal cartilage to find the structural changes to the DSC data.

Acknowledgements

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