INFLUENCE OF ENDOTRACHEAL CUFF PRESSURE ON TRACHEAL MICROCIRCULATION IN INTUBATED CRITICALLY ILL PATIENTS

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Abstract

Problem: Tracheal ischaemic lesions are common in critically ill intubated patients due to unregulated endotracheal cuff pressure during intubation. A high endotracheal cuff pressure disturbs the microcirculation which leads to ischaemia. Clinicans in the Radboud University Medical Center created an adjusted Tonocap that may be able to evaluate the tracheal microcirculation during intubation by measuring the amount of CO_2.

Aim: This research contains three parts. Part 1 is to determine whether the adjusted Tonocap has the ability to adequately measure the CO_2 concentration. To make the adjusted Tonocap potential adaptable in clinical setting, the endotracheal cuff pressure needs the be reduced. Part 2 is to find a gold standard for measurement of the microcirculation in the trachea. This gold standard has to be used in future in a testing animal to validate whether the adjusted Tonocap measures the right CO_2 content in vivo. Part 3 is about application of the adjusted Tonocap and the gold standard.

Method: Part 1: Measurements were done in an airtight box with variable CO_2 concentrations. Part 2: A literature research is done to determine which techniques are suitable as a gold standard. Part 3: An advice is given for further implementation and application of the gold standard and the adjusted Tonocap.

Results: Part 1: The adjusted Tonocap measures a lower concentration of CO_2 (deviation lies between 1.8% and 10.7%) compared with a CO_2 sensor (Anagas CD98). Multiple experiments are done to reduce the cuff pressure by enlarging the volume of the cuff and reducing the amount of gas. Part 2: 39 techniques were found to measure microcirculation. After evaluating, these techniques, Laser Doppler Flowmetery and Sidestream Dark Field remained as potential gold standards. Part 3: Measurements must be performed with the adjusted Tonocap and O2C together in the trachea.

Conclusion: Part 1: The adjusted Tonocap measures the CO_2 concentration and is expected to have the ability to measure the microcirculation in the tracheal wall. To lower the cuff pressure a potential solution is seen in enlarging the volume of the cuff. Part 2: Laser Doppler Flowmetry is the best technique for measurements of the microcirculation in the tracheal wall. The O2C device is recommended for validation of the adjusted Tonocap because it uses Laser Doppler Flowmetry. Part 3: It is likely that both techniques fit together in the trachea. Combination of the outcomes makes validation of the adjusted Tonocap possible.

Preface

This report is the result of our multidisciplinary assignment, the final project of the Bachelor Technical Medicine at the University of Twente. The past ten weeks, we worked hard to get answers on all the questions that we and our supervisors had during this project. We would like to thank our medical supervisors from the Radboud University Medical Centre, Joris Lemson and Simone Timman, for coming up with this interesting assignment and for their help during this bachelor thesis. We also would like to thank our technical supervisor from the University of Twente, Lex van Loon, for his useful feedback and critical questions. Thanks to Paul ter Braak and Anne Leferink for using their lab and giving us (almost) all devices that we needed for our measurements. Last but not least, we would like to thank Nicole Rommens for all the meetings we had together and for giving us useful feedback on our personal development.

Enjoy reading,

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Contents

Reading guide

This reading guide provides a short overview of this research. The global contents are described for each section. This research is introduced in chapter 1. In this chapter relevant anatomical and technical background information for this research is described. The problem, aim and research question are defined in chapter 2. Chapter 3 is devoted to the method and chapter 4 to the results of this research. Chapter 5 includes the discussion of the research and finally, the research is concluded in chapter 6.

List of abbreviations

CO .... Carbon monoxide

CO$_2$ .... Carbon dioxide

Hb .... Hemoglobin

HCO$_3^-$ .... Bicarbonate

HI .... Heterogenity index

IDF .... Incident Dark Field

LDF .... Laser Doppler Flowmetry

LEDs .... Light-emitting diodes

MFI .... Microvascular flow index

N$_2$O .... Nitrous oxide

NIRS .... Near Infrared Spectroscopy

O$_2$ .... Oxygen

O2C .... Oxygen to See

OPS .... Orthogonal Polarization Spectral imaging

PCD .... Perfused capillary density

PCO$_2$ .... Partial pressure of CO_2

pHi .... Intramucosal pH

PPG .... Photopulse Plethysmography

PVD .... Perfused vessel density

PPV .... Proportion of perfused vessels

PW HFD .... Pulsed Wave High Frequency Doppler

Radboudumc .... Radboud University Medical Centre

RBCs .... Red blood cells

rHb .... Relative amount of hemoglobin

SDF .... Sidestream Dark Field

SLD .... Scanning Laser Doppler

Chapter 1: Introduction

1.1 Post-intubation tracheal complications

Endotracheal intubation is used in critically ill or anesthetized patients to open their airways and facilitate ventilation, support breathing and give medicine. During endotracheal intubation, a plastic (polyvinylchloride) tube with a 'balloon' is inserted into the trachea. When the balloon is placed just above the far end of the tube, it is inflated to form a cuff. This endotracheal cuff prevents gastric contents to pass in the lungs and respiratory gases to leak to the abdomen. Common complications of endotracheal intubation (Figure 1.1) are laryngeal damage, ischaemia and late subglottic- and tracheal stenosis around the cuff site.[60] Two main causes for these complications are that it is unknown what endotracheal cuff pressure is best suitable per patient and that the cuff pressure is not measured during endotracheal intubation.[69] An endotracheal cuff pressure up to 30 mmHg (40 cmH$_2$O) [69] exceeds the mucosal capillary pressure, which can cause occlusion of the vessels.[16][60] Occlusion of the capillary vessels leads to ischaemia in the surrounding tissue. Long standing ischaemia can lead to ulceration and chondritis of the tracheal cartilages. These lesions will heal by fibrosis, which leads to a progressive tracheal stenosis.[60]

Post intubation stenosis is an important cause of an acquired tracheal obstruction. In 1981, a prospective study showed that 11% of 150 critically ill patients intubated with a 'high volume low pressure cuffed' endotracheal tube developed tracheal stenosis of 10-50% of their tracheal diameter at the cuff site. This stenosis developed within two weeks after extubation.[62] A more recent study that included 96 critically ill adult patients found a higher incidence of tracheal ischaemic lesions (83%) after 24 hours of extubation.[16] Of the patients, 38% had a tracheal ischaemia score with a score 5/10 which means that the patient had ischaemic lesions, ulcer or a tracheal rupture. All complications were found around
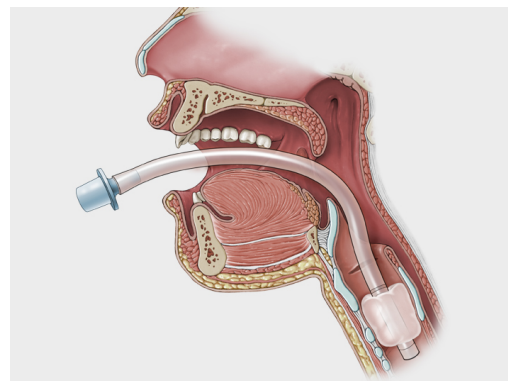
Figure 1.1: Endotracheal intubation [47]

the cuff. In clinical practice, it may take two weeks up to three months before a patient is diagnosed with post-intubation stenosis, because they usually remain asymptomatic until the trachea has stenosed 30% of its original diameter.[16]

The staff of the intensive care unit of Radboud University Medical Centre (Radboudumc) in Nijmegen developed the idea to apply tonometry during endotracheal intubation to measure the microcirculation and to regulate the endotracheal cuff pressure. Therefore, they created an adjusted Tonocap that is theoretically able to evaluate the tracheal microcirculation by measuring carbon dioxide (CO_2). However, this adjusted Tonocap has not been validated yet. In this paper the possibilities of this technique are reviewed and experiments are done to prove the applicability of tonometry in this setting. Before implementation of the technique validation is necessary. A systematic review is done to find a gold standard in order to make validation of tonometry in the trachea possible.

1.2 Anatomic background

1.2.1 Histology

The trachea is part of the conducting part of the respiratory system and consists of 20 U-shaped tracheal cartilages of hyaline which are coated by perichondrium. The two ends of the U-shape are dorsally connected by a membrane, which forms the dorsal membranous wall. This dorsal membrane is flexible and therefore allows stretching of the esophagus.[25][33] The trachea consists of five layers, from the inner to the outer side: 1) the respiratorium epithelium, 2) the lamina propria, 3) the submucosa, 4) the U-shaped hyaline cartilage and 5) the adventitia.[19] (Figure 1.2)
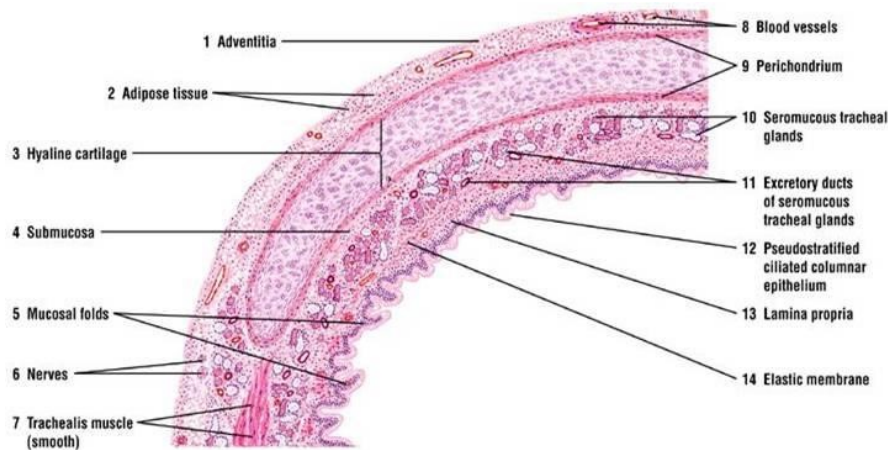
Figure 1.2: A transversal section of the trachea[19]

1.2.2 Blood supply

Blood vessels are present in the lamina propria and the adventitia.[19][33] Blood enters the tracheal wall via the lateral tissue pedicles throughout the trachea in a segmental fashion. The blood supply consists of three arteries: 1) the a. trachealis, 2) the a. oesophagealis and 3) the a. bronchialis. The a. trachealis and a. oesophagealis provide blood to the proximal part of the trachea. The a. trachealis nourishes the lateral side of the trachea. The branches of the a. trachealis continue to the ventral part of the trachea. The submucosal capillary plexus arises there. The a. oesophagealis nourishes the dorsal part of the trachea. The distal part of the trachea is vascularised by the bronchial artery.[17] (Figure 1.3)
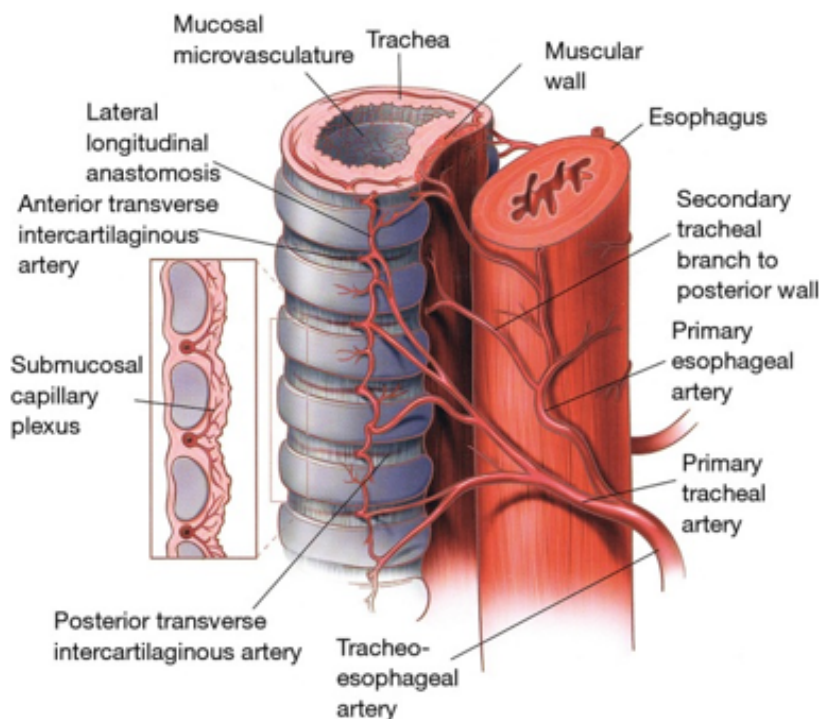
Figure 1.3: A transversal section of the trachea[19]

1.3 Technical background

1.3.1 Effect of unregulated endotracheal cuff pressure

During endotracheal intubation, it is recommended to maintain an endotracheal cuff pressure between 15 and 22 mmHg (20 and 30 cmH$_2$O). An endotracheal cuff pressure less than 15 mmHg (20 cmH$_2$O) may promote drainage of pharyngeal secretion and air leak. A pressure of the endotracheal cuff of more than 22 mmHg (30 cmH$_2$O) may exceed

the capillary perfusion pressure which varies from 18 to 26 mmHg (25 to 35 cmH$_2$O). This capillary perfusion pressure is defined as the difference between the capillary pressure and the tissue pressure. At the arterial side a positive capillary perfusion pressure is present. Therefore fluid together with oxygen O$_2$ can leave the bloodstream and nourish the tissue. At the venous side a negative capillary perfusion pressure is present, so fluid together with CO$_2$ can leave the tissue and return to the bloodstream.[41] A high cuff pressure increases the tissue pressure, whereby fluid and O$_2$ can not leave the bloodstream and the tissue will be less nourished. This can cause lesions of the mucosa and can lead to ischaemia.[14][38][49] Ischaemia can lead to necrosis and results in loss of mucosa and the structural integrity of the tracheal wall. The effect of endotracheal cuff pressure on the mucosal wall differs between the dorsal and ventral side. The dorsal membranous tracheal wall is more distensible than the cartilaginous ventral wall. Therefore, cuff-related tracheal damage is most severe in the ventral wall of the trachea.[13]

Figure 1.4a and b show the tracheal mucosal blood flow in its anterior and posterior wall using different cuff pressures. These pictures were obtained using an endoscopic photographic technique.[67]

(a) Anterior   (b) Posterior
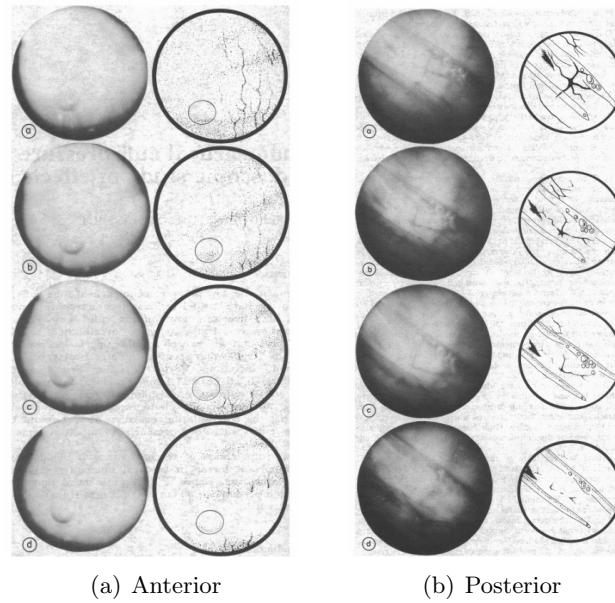
Figure 1.4: Effect of cuff pressure on blood flow in the anterior/posterior mucosa. a:22 mmHg b: 29 mmHg c:37 mmHg d: 44-74 mmHg. Pictures were obtained using an endoscopic photographic technique.[67]

- Pressure of 22 mmHg (30 cmH$_2$O): The anterior mucosa over the tracheal rings becomes less pink in the intercartilaginous areas. Posterior vessels in the submucosa overlying the tracheal rings are normal.

- Pressure of 29 mmHg (40 cmH$_2$O): The anterior mucosa is pale, some vessel in the submucosa are reduced. Vessels in the submucosa overlying the tracheal rings are normal. Posterior vessels are normal.

- Pressure of 37 mmHg (50 cmH$_2$O): The anterior mucosa becomes blanch and vessels are not visible. The intercartilaginous mucosa is normal. The posterior mucosa becomes blanch and the vessels are more attenuated.

- Pressure of 44-74 mmHg (60-100 cmH$_2$O): The anterior mucosa over the tracheal rings remained blanch, no vessels are visible in the overlying mucosa. The intercartilaginous mucosa is normal. The posterior vessels become more progressively attenuated. There is total obstruction of the blood flow to the mucosa overlying the tracheal cartilages.[49]

1.3.2 Tonocap as possible solution

Clinicians of Radboudumc created a tube with a tonometry balloon (Figure 1.5) that is able to evaluate the effect of the endotracheal cuff pressure on the endotracheal microcirculation by measuring CO$_2$. This cuff is permeable to CO$_2$. The adjusted Tonocap (Figure 1.6) would be able to measure CO$_2$ concentration. Evaluation of CO$_2$ concentration in tissue affords a measurement of tissue perfusion. CO$_2$ accumulates during a period of reduced perfusion. Thus, an increase in CO$_2$ concentration can be used to evaluate the adequacy of tissue perfusion.[78] Although the Tonocap has not yet been used to measure the microcirculation in the trachea wall, it has shown to be effective in the stomach.

Figure 1.5: The adjusted tube, a normal endotracheal tube combined with a tonometry balloon.

Figure 1.6: Set up of the adjusted Tonocap

1.3.3 Air tonometry with the Tonocap

The technique of the Tonocap is based on air tonometry and is used to measure CO_2 in the gastric lumen. The Tonocap uses the partial pressure of CO_2 (PCO_2) to determine the capillary perfusion. This measured PCO_2 corresponds to the PCO_2 of the lumen and the mucosa.[5][22] Because tissue CO_2 diffuses from regional blood vessels into the lumen of hollow visceral organs, the luminal PCO_2 corresponds to tissue PCO_2. This PCO_2 reflects the balance between metabolic CO_2 production and CO_2 removal by perfusion and blood flow. [33] Hypoperfusion and mucosal acidosis both lead to an elevated PCO_2 compared to the arterial PCO_2 (PCO_2 gap).[5] The following equilibrium shows that mucosal acidosis will lead to an elevated PCO_2.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2\,H^+ + CO_3^{2-}$$
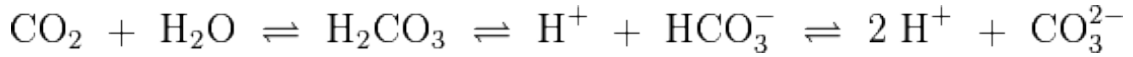
After positioning the tube, air is injected into the Tonometer's balloon. After a period of equilibration, the balloon contents can be withdrawn to analyse the PCO_2 using an infrared analyser.

Infrared gas analysers used in the Tonocap usually have two chambers: a measurement and a reference chamber. Infrared light is emitted from a source on one end of the chamber and passes through a serie of chambers that contains given quantities of the various gases in question.[1] The infrared chamber is a CO_2 and nitrous oxide (N_2O) dual-path photometer. The infrared light beam passes through a measuring chamber that contains the gas that should be analysed. It also passes a reference chamber which is filled with carbon monoxide (CO) and N_2O. The measurement is done by determining the ratio between the two light intensities.[22][64] The Tonocap monitor provides an automated measurement of PCO_2. Results of sampling may be viewed as CO_2 gaps. These results may then be viewed as trends over selected time periods.[22]

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1.3.4 Outcome of the Tonocap

The Tonocap gives two important outcomes, regional PCO_2 $PrCO_2$ and intramucosal pH (pHi).

$PrCO_2$: Regional PCO_2 is the partial pressure of CO_2 in the lumen. The $PrCO_2$ value shows the balance between the CO_2 production and its removal by mucosal perfusion and alveolar ventilation.[80] In this research the $PrCO_2$ will show the partial pressure of carbon dioxide in the tracheal mucosa.[61]

The Tonocap is able to measure values of $PrCO_2$ between 0-76 mmHg (CO_2 concentrations between 0-10%) and in an extended range from 76-106 mmHg (CO_2 concentrations between 10-14%). The Tonocap has an equilibrium period of ten minutes and measures the $PrCO_2$ after equilibration. The $PrCO_2$ is above normal when it exceeds 61 mmHg (CO_2 concentration of 8%).[75]

pHi: To calculate the pHi two assumptions have to be made. The first assumption is that the measured $PrCO_2$ by the Tonocap approximates the intramucosal PCO_2. The second assumption is that intramucosal and arterial bicarbonate (HCO_3^-) measurements are the same. The HCO_3^- concentrations are measured from the blood. With these values, the pHi can be calculated using the Henderson formula: $pHi = 6, 1 + \log 10 [HCO_3^-]/[a * PCO_2]$

a=0.03, the solubility factor of CO_2 in plasma. A decreased pHi reflects an increased anaerobic metabolism or a low flow state and correlates with mucosal ischaemia. A pHi <7.32 reflects acidosis.[81]

In general, measurement of the $PrCO_2$ gives a more accurate reflection than pHi of the micro-circular perfusion in vivo.[81]

Chapter 2: Problem definition and research question

2.1 Problem definition

Tracheal ischaemic lesions are common in critically ill intubated patients due to unregulated endotracheal cuff pressure during intubation. A high endotracheal cuff pressure disturbs the microcirculation which can lead to ischaemia. Clincans in the Radboudumc created an adjusted Tonocap that should be able to evaluate the tracheal microcirculation during intubation by measuring the amount of CO_2. However, this adjusted Tonocap has not yet been validated. Firstly, it is not known whether this adjusted Tonocap can adequately measure CO_2 concentration in this environment. Secondly, a validation by a gold standard is needed to determine the efficacy of the adjusted Tonocap for measuring the microcirculation of the tracheal wall. This gold standard needs to be reviewed in literature.

2.2 Aim

There are three main aims in this study. The first aim is to determine whether the adjusted Tonocap is able to measure the CO_2 concentration in vitro. This could predict whether the adjusted Tonocap is able to measure the microcirculation in the tracheal wall accurately in vivo. The second aim is to determine the gold standard for measuring microcirculation in the tracheal wall, which can be used for validation of the adjusted Tonocap in vivo.

2.3 Research question

The research question of this study is:

How can air tonometry be used to determine the effect of endotracheal cuff pressure on the tracheal microcirculation during endotracheal intubation?

In addition, the following sub questions are compiled:

- How can the adjusted Tonocap measure the microcirculation in the tracheal wall?

- What test setup must be used to evaluate whether the adjusted Tonocap is functional for measuring CO_2?

- Which technique can be used as a gold standard to validate the adjusted Tonocap to measure the microcirculation in the tracheal wall?

  – Which techniques are currently available to measure the microcirculation?
  – Which of these techniques is most suitable for measuring the microcirculation in the tracheal wall?

Chapter 3:   Method

This research can be divided into three parts. The goal of part 1 is to check whether the Tonocap measures the right CO_2 concentration. The aim of part 2 is to find a gold standard to validate the Tonocap. Part 3 will focus on combining part 1 and part 2.

3.1   Part 1: Measurements of the Tonocap

It is unclear whether the combination of an endotracheal tube with the original Tonocap (which is the adjusted Tonocap) is able to measure the CO_2 content accurately. This will be verified in four steps.

3.1.1   Step 1: Measurements in an environment with a constant CO_2 concentration

The first measurement is performed in an incubator (Binder) with a constant CO_2 concentration of 5%. When the adjusted tube is placed in this incubator, it is possible to compare the outcome of the adjusted Tonocap with the constant CO_2 concentration. Three measurements will be done to confirm whether the Tonocap is able to measure a concentration of 5%. When the adjusted tube is placed in this incubator, it is possible to compare the outcome of the Tonocap with the constant CO_2 concentration. Three measurements will be done to confirm whether the Tonocap is able to measure a concentration of 5%. Besides, a CO_2 sensor (Anagas CD98) will be placed in the incubator to measure the CO_2 concentration. When these measurements are in accordance with the incubator, the CO_2 sensor can be used for further measurements.

3.1.2   Step 2: Evaluation of the air tightness of the box

To determine whether the adjusted Tonocap is able to measure variable CO_2 percentages, an airtight setup (box) has to be designed in which the CO_2 percentage can be regulated, see Figure 3.1. The box must contain three inputs for: the CO_2-sensor, the CO_2-pump and the adjusted Tonocap. The inputs will be attached with silicone sealant. The box is airtight and the CO_2 will be pumped in, therefore the pressure in the box raises. A pressure valve is needed to prevent loosening of the lid of the box, when the pressure is too high.

It remains unclear whether the fabricated box is completely airtight. To test the air tightness of the box, CO_2 will be inflated in the box and meanwhile the CO_2 concentration will be measured. Therefore, the CO_2 sensor will be used to measure the CO_2 concentration every minute. When the concentration does not decrease, the box is airtight. When the concentration decreases during the first ten minutes, the box is not airtight and steps must be taken to make the box more airtight.
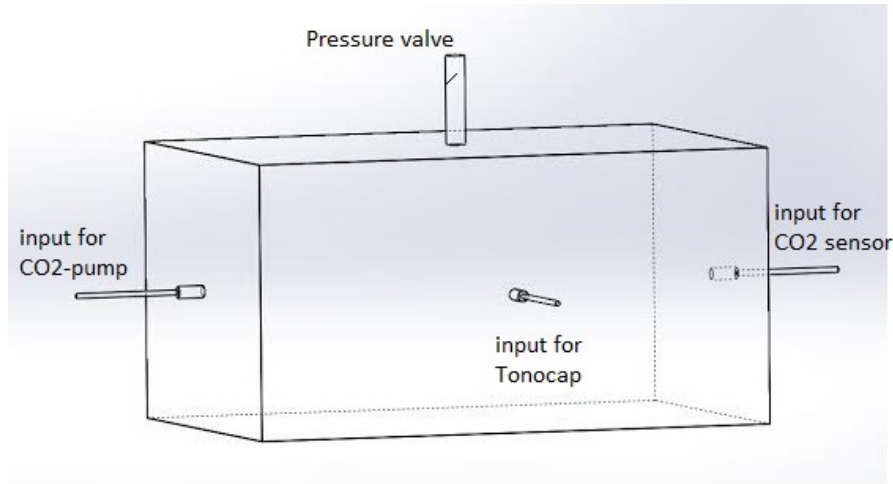
Figure 3.1: Design of the airtight box

3.1.3 Step 3: Measurements in an environment with a variable CO_2 concentration

The Tonocap should be capable to measure variable CO_2 concentrations in the trachea. All measurements are performed with usage of the airtight box. Every measurement with the Tonocap takes ten minutes. During these ten minutes, an average of three measurements (at start, after five minutes and after ten minutes) with the CO_2 sensor is made to compare the measurements of the Tonocap with the CO_2 sensor.

The values of both measurements methods will be analysed with Statistical Package for the Social Science (SPSS). A paired T-test will be used with a significance level (α) of 0.05.

3.1.4 Step 4: Adjustments of the cuff pressure

The Tonocap causes a cuff pressure around 65 mmHg [75], but a cuff pressure of 20-30 mmHg is accepted during endotracheal intubation. Therefore, a system will be created to decrease this cuff pressure of 65 mmHg. Subsequently, CO_2 concentrations

are measured to evaluate whether the adjusted Tonocap also functions at lower cuff pressures around 20-30mmHg.

The Tonocap inflates a gas, assumed as dry air, into the cuff. The ideal gas law describes an approximation of the behavior of this gas in the system. The ideal gas law is P*V = n*R*T.

- P = pressure in Pascal (N/m^2): the cuff pressure.

- V = volume in m^3 : volume of air in the cuff.

- n = amount of gas in moles: the molecular weight of air is 28.97g/mole.

- R = the ideal universal, gas constant: 8.31 J $K^{-1}mol^{-1}$

- T = temperature of the gas in Kelvin

The variables in this equation are the volume the amount of gas, the temperature and the pressure. To lower the pressure in the cuff, V should be increased or n or T should be decreased. Several experiments will be done based on two methods, decreasing the amount of gas (n) and increasing the volume (V) of the cuff.

First method: Reduce the amount of gas

By reducing the amount of gas (n), the cuff pressure will decrease. Next to the adjusted tube, a venting system will be connected to the Tonocap. The venting system exists of a manometer (to measure the pressure in the system) and a syringe (for drainage of gas from the cuff). A tap between the Tonocap, the adjusted tube and the venting system allows opening and closing of the different airways. To reduce the amount of gas, the first step is to open the airway from the Tonocap to the adjusted tube. (Figure 3.2) The cuff pressure will raise above the desired pressure of 20-30 mmHg.
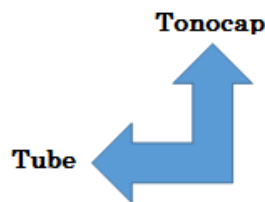
Figure 3.2: Step 1, tap opened in two directions

The second step is to open the tap in all three directions. (Figure 3.3) Because the pressure needs to be reduced to an acceptable pressure for trachea, which is between 20-30 mmHg (0.027-0.040 Bar). A certain amount of gas will be drained by the syringe and after that the venting system will be closed. After an equilibration period of 10 minutes, the Tonocap will analyse the gas sucked from the adjusted cuff.
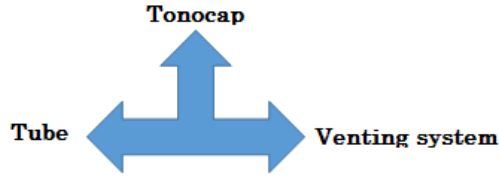
Figure 3.3: Step 2, tap opened in all three directions

Second method: Enlarge the volume of the cuff

The cuff pressure can also decrease by enlarging its volume. Currently, there are no bigger adjusted tonometry cuffs available than the one that is used in this research. Therefore, this measurement will be carried out with a normal endotracheal intubation tube with cuff. When the Tonocap is able to analyse the gas from this cuff, a larger cuff could be a possible solution. It must be taken into account that the normal intubation cuff is not CO_2 permeable. This means that the Tonocap will not measure correct concentrations of CO_2.

3.2   Part 2: Gold standard

If the adjusted Tonocap has shown to be effective in measuring CO_2 in predetermined conditions ex vivo, which means that it can measure with a maximal deviation of 10%, the adjusted Tonocap could be validated in vivo. First, a gold standard needs to be found which can validate the adjusted Tonocap in measuring the microcirculation in the trachea.

3.2.1   Step 1: First criteria

To find a gold standard for measuring microcirculation in the tracheal wall several criteria are established.

- Applicability

- Validation of the technique

- Size

- Depth of measurement

- Continue or sampled measurement and time of measurement.

Applicability
The trachea of an animal will be opened with an incision, this will take place in an operation room. The gold standard needs to be directly applicable at the bedside of the

animal in the operation room.

Validation of the technique
To be used as a gold standard, the technique should already have been validated.

Size
Probably a goat will be used to validate the adjusted Tonocap. The size of the trachea of a goat is similar to a human trachea. [72] The standards of the human trachea are used to determine the usable space in the trachea to perform the measurements. A human trachea has a length of 110 mm an average diameter of 18.3 mm [12] and a circumference of 57.5 mm. The trachea will be intersected longitudinal, this makes it possible to open up the trachea. When the trachea is opened a width of 57.5 mm is available for placement of the technique. The available length is the total length (110 mm)[74] minus the necessary space for placement of the cuff, assumed as (50 mm). This results in a usable length of 60mm. Techniques with materials exceeding these sizes are considered as not useful.

Depth of measurement
The tracheal wall comprises a few layers. The total wall has a thickness of 1-3 mm.[9] Blood vessels are present in the lamina propria and the adventitia. The measurements should therefore be performed in these layers. These layers are positioned 0.0 - 0.1 mm under the lumen (lamina propria) and 1.0-1.2 mm under the lumen (adventitia). [59][19] A technique would be most functional if it measures from the lumen with a depth between 0.0 and 1.2 mm.

Continue or sampled measurement and time of measurement.
One measurement cycle of the adjusted Tonocap takes 10 minutes.[20][32] The outcome of the adjusted Tonocap is an average CO_2 value which has been observed over these 10 minutes. Therefore, a continuous measurement by the gold standard is not necessary. A technique would be suitable when the measurement occurs over a period of 10 minutes. It would be more accurate when multiple measurements are performed within these 10 minutes. This makes continuous and sampled measurement both suitable.

A literature review will be performed to find the gold standard. The review will start with an overall search to find techniques that measure microcirculation. After completing this search, unsuitable techniques are excluded with the list of criteria.

3.2.2 Step 2: Evaluation of the remaining techniques

After considering the identified techniques with the above mentioned selection criteria, there might remain several techniques as potential gold standards. To find out which of these techniques can be used as a gold standard, the following questions have to be answered:

1. Is the technique mentioned as a gold standard in literature?

2. For which applications is the technique suitable?

3. Does the technique need adjustments for usage in the trachea?

4. Is the technique still in use and is it used for a longer period?

5. Is the technique likely to be functional for measuring microcirculation in the tracheal wall?

3.2.3   Step 3: Recommended techniques

With answers on the questions above, it should be possible to recommend a technique as gold standard. These techniques will be examined in detail.

3.2.4   Step 4: Recommended devices

These recommended techniques are applied in certain devices. Therefore a selection of the best devices will be made.

3.3   Part 3: Application of the adjusted Tonocap and the gold standard for validation

In this research, part 1 and part 2 will be carried out independently of each other. However, it is of great importance to combine these two parts because this will make it usable for the clinicians at Radboudumc.

In this part a consideration will be given about a set-up to validate the Tonocap. Next to it the outcomes of the adjusted Tonocap and the gold standard have to be compared.

Chapter 4:   Results

4.1   Part 1: Measurements of the Tonocap

4.1.1   Step 1: Measurements in an environment with a constant CO_2 concentration

Three measurements have been done in the incubator with a constant CO_2 concentration (Table 4.1). In all three measurements, the adjusted Tonocap showed a 0.5% lower CO_2 concentration than the incubator. This difference reflects a systematic deviation of 10%. The measurements of the CO_2 sensor are also lower than those of the incubator with a mean deviation of 2.7%. The deviation can be calculated with the following formula: $value of incubator - measured value/value of incubator * 100\%$.

	Incubator (%)	CO_2 sensor (%)	Adjusted Tonocap (%)
Measurement 1	5.0	4.8	4.5
Measurement 2	5.0	4.9	4.5
Measurement 3	5.0	4.9	4.5

Table 4.1: Measurements in an incubator with 5% CO_2

4.1.2   Step 2: Evaluation of the air tightness of the box

During measurement one, there is an absolute difference of 6.2% between the first and last measurement. During measurement two, after improvement of the fabricated box, the deviation decreased to +0.7%. The measurement after 15 minutes had the largest deviation. After the second measurement the CO_2 supply was locked after inflating CO2 in the box. During the third and fourth measurements the CO_2 concentration decreased to 8.5% and 5.9%. At the end of measurement three there was an absolute deviation of +0.2% CO_2 and at the end of measurement four, an absolute deviation of -0.3%. Results are shown in table 4.2.

Time (minutes)	CO$_2$ concentration (1)(%)	CO$_2$ concentration (2)(%)	CO$_2$ concentration (3)(%)	CO$_2$ concentration (4)(%)
0	30.9	9.4	8.3	6.2
1	30.7	9.2	8.3	6.1
2	29.6	9.2	8.3	
3	28.9	9.2		6.0
4	28.8	9.2	8.2	
5	28.5	9.2	8.2	6.0
6	27.6	9.2		
7	27.2	9.2	8.1	
8	26.5	9.2		5.6
9	26.3	9.2		
10	26.0	9.2	8.1	5.7
15	24.7	10.1	8.5	5.9
Absolute deviation	-6.2	+0.7	+0.2	-0.3

Table 4.2: CO$_2$ concentration in the fabricated air tight box

4.1.3 Step 3: Measurements in an environment with a variable CO$_2$ concentration

During 18 measurements of 10 minutes, the CO$_2$ concentration was measured by the adjusted Tonocap and CO$_2$ sensor. As visible in table 4.3, in measurements 1 and 10 are not valid because the adjusted Tonocap measured a higher CO$_2$ concentration than the CO$_2$ sensor did. Measurement 2 shows a deviation out of range compared to the other measurements. Measurements 8 and 9 are not valid, because the three measurements of the CO$_2$ sensor differed more than 3%.

The remaining 13 valid measurements can be used to evaluate whether the adjusted Tonocap can measure different CO$_2$ concentrations. CO$_2$ concentrations measured with the adjusted Tonocap were 1.8% to 10.8% lower than those measured with CO$_2$ sensor. The mean deviation was 6.1%. Results are shown in table 4.3.

Measurement	Adjusted Tonocap (%)	Average CO$_2$ sensor (%)	Relative deviation (%)
1	3.1	2.28	-36.0
2	1.4	3.43	59.2
3	3.5	3.6	2.8
4	6.7	7.2	6.9
5	12.0	12.6	4.8
6	12.8	13.9	7.9
7	12.5	13.0	3.5
8	13.2	13.4	1.5

9	9.6	8.8	-9.1
10	8.5	6.8	-25.0
11	3.2	3.5	8.6
12	3.5	3.7	5.4
13	4.1	4.4	6.8
14	8.3	9.3	10.8
15	8.7	9.6	9.4
16	10.5	11.0	4.5
17	5.5	5.6	1.8
18	2.1	2.22	5.4

Table 4.3: CO_2 concentrations after ten minutes measured by the adjusted Tonocap and CO_2 sensor. The average CO_2 concentration measured by the sensor was calculated by the mean of the measurements at t=0 min, t=5 min and t=10 min.

Figure 4.1 shows the measured CO_2 concentrations by the adjusted Tonocap against the realistic CO_2 concentrations (measured by the CO_2 sensor). The trend line represents the realistic CO_2 concentrations and the dots represent the CO_2 concentrations measured by the adjusted Tonocap. All measurements of the adjusted Tonocap lie below the trend line.
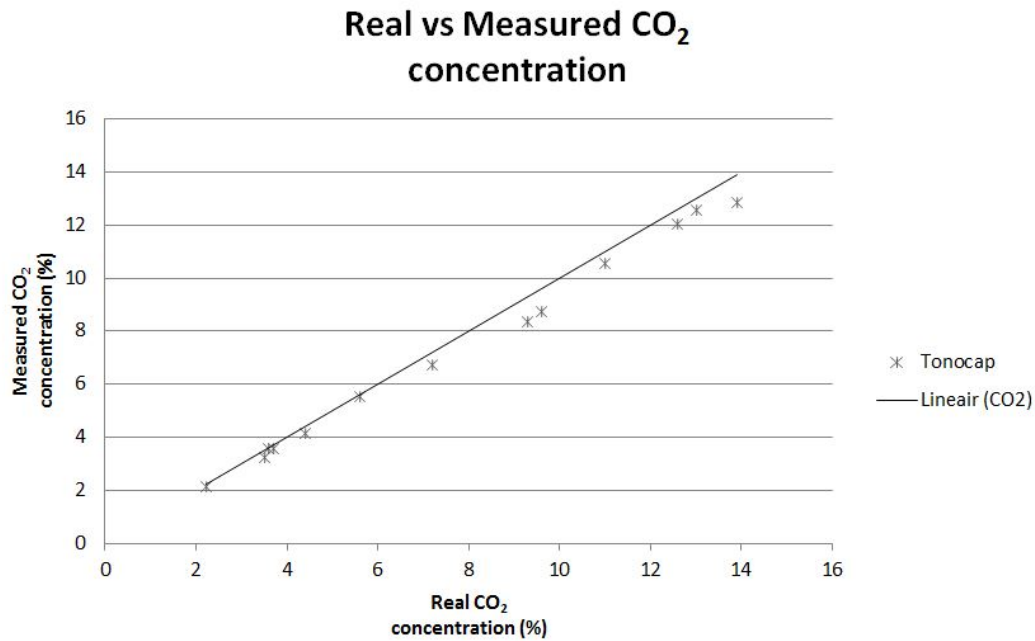
Figure 4.1: CO_2 concentration measured by the adjusted Tonocap set out against the CO_2 concentration measured by the CO_2 sensor.

After step three, a statistical analysis of the results of step one was performed. The

results of step one and three have been analysed with SPSS. A paired T-test was used to test whether significance of the differences between both measurements methods could be found. The results in figure 4.2 show that the CO_2 concentrations measured by the CO_2 sensor were significantly higher than those measured by the adjusted Tonocap.

Paired Samples Test

| | | Paired Differences | | | | | | | |
| | | | | | 95% Confidence Interval of the Difference | | | | |
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	Sensor_incubator - Tonocap_incubator	,36667	,05774	,03333	,22324	,51009	11,000	2	,008
Pair 2	Sensor_fabricated_box - Tonocap_fabricated_box	,47846	,34258	,09502	,27144	,68548	5,036	12	,000

Figure 4.2: Statistical analysis of step 1 and step 3 done with a paired T-test.

The relative deviation has been calculated with: $(valueCO2sensor - valueTonocap)/valueCO2sensor * 100\%$. Figure 4.3 shows that the relative deviation does not change with certain concentrations of CO_2, as can be seen with the relatively horizontal trend line, corresponding to y = 0.0853x + 5.4162. The corresponding coefficient of determination is 0.0179.

Figure 4.3: Relative deviation between the CO_2 sensor and the adjusted Tonocap set out against the CO_2 concentration.

4.1.4   Step 4: Adjustments of the cuff pressure

First method: Reduce the amount of gas

Option 1, reduce the amount of gas
By placing a manometer in the system, it is possible to measuring the pressure. After filling the cuff with gas by the Tonocap the path to the manom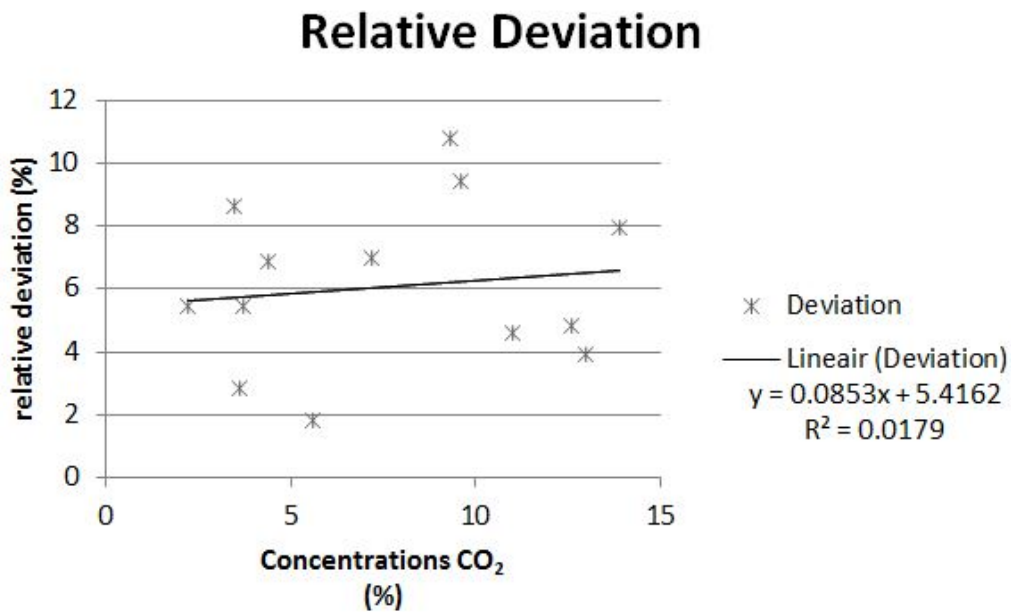eter was opened. The amount of gas that is deflated by the Tonocap is 5-8ml. The highest pressure measured was 0.11 bar. The pressure was reduced by the syringe to 0.03 bar (22.5mmHg), which is accepted in the trachea. Analysis of the CO_2 concentrations using this cuff pressure was performed while the opening to the venting system was (syringe and manometer) closed (Table 4.4).

Measurement	Pressure in the cuff (bar)	CO_2 concentration adjusted Tonocap (%)	CO_2 concentration incubator (%)
1	0.03	0.1	5
2	0.03	0.2	5
3	0.07	0.1	5
4	0.08	0.5	5
5	0.08	0.2	5

Table 4.4: Measurement of the CO_2 concentration with a reduced cuff pressure.

The Tonocap measured a CO_2 concentration that is lower than the real CO_2 concentration in the incubator. It is notable that even at the same cuff pressure a different CO_2 concentration in measured. During all these measurements the Tonocap gave the signal catheter pressure < minimum.

Option 2, reduce the amount of gas.
The pressure in the cuff should be reduced to 0.03 bar. Therefore, a pressure regulator was placed between the adjusted Tonocap and the catheter. The pressure regulator cuts of the flow if it rises above 0.03 bar. The high pressure enters the valve by the inlet gauge as shown in figure 4.4. The high pressure pushes the diaphragm which closes the inlet valve. This prevents the inlet of more gas and the outflow of air with a high pressure. The volume and pressure coming from the Tonocap is around 6 ml and causes a maximal pressure of 0.11 bar in the system. The pressure in the regulator was set at 0.03 bar, because this is in accordance with a maximal pressure of 22.5 mmHg.

No results were gained with this setup because the cuff did not fill with gas.
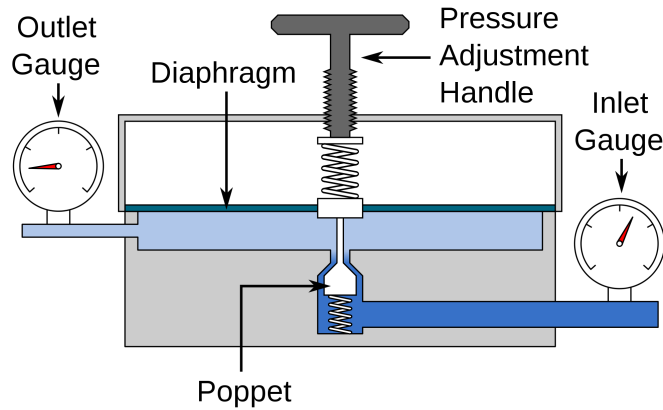
Figure 4.4: Reduce the amount of gas with a pressure regulator.

Option 3, reduce the amount of gas

Thirdly, the total volume was manually divided over the cuff and the syringe. Gas was released from the adjusted Tonocap by opening the tap to the syringe. A part of the total volume in the syringe was blown in the cuff. During analysing both paths, the syringe and the adjusted cuff, were opened. The adjusted cuff was placed in the incubator with a 5% CO_2 environment and the syringe was filled with dry air (0.013% CO_2). Because the volumes and the CO_2 percentages of the air in the injector and the cuff were known, it could be calculated whether the adjusted Tonocap has measured the correct concentration PCO_2. This can be calculated with the following formula:

$Expected CO_2 = ((Volume cuff/totalvolume*5*100)+Volume injector/totalvolume*100*0.013))/100$

Measure-ment	Total volume (ml)	Volume cuff (ml)	Volume syringe (ml)	Expected CO_2 (%)	Measured CO_2 (%)	Relative deviation (%)
1	4.9	0.9	4	0.93	11.38	-1164.4
2	5.5	1.4	4.1	3.7	5.46	-48.6
3	5.5	1.4	4.1	3.7	5.07	-35
4	5.5	2.0	3.5	1.8	0.9	50
5	5.5	2.0	3.5	1.8	1.1	31.25
6	5.5	2.0	3.5	1.8	2.7	-80
7	5.7	3.7	2.0	3.2	2.4	27.3

Table 4.5: expected CO_2 value calculated by dividing the gas over the cuff and the syringe.

Table 4.5 shows that the measured CO_2 differs from the expected CO_2. After opening the tap to the adjusted cuff and closing the tap to the syringe, the deviation was smaller than during opening the tap in the direction of the syringe and the cuff. (Table 4.6)

Measurement	CO$_2$ incubator (%)	CO$_2$ cuff (%)	Deviation (%)
1	5	4	20
2	5	4.5	10
3	5	4.5	12
4	5	4.2	16
5	5	4.1	18

Table 4.6: CO$_2$ concentration measured during analysis of the air out of the adjusted cuff. The tap openend only in the direction of the cuff

After opening the tap to the syringe and closing the tap to the cuff, the deviation was bigger than during opening the tap in the direction of the syringe and the cuff (Table 4.7).

Measurement	CO$_2$ room air(%)	CO$_2$ syringe (%)	Deviation (%)
1	0.013	1.6	-12207.7
2	0.013	0.9	-6823.1
3	0.013	0.7	-5284.6
4	0.013	0.6	-4515.4
5	0.013	0.5	-3746.2
6	0.013	0.4	-2976.9
7	0.013	1.7	-12976.9
8	0.013	1.5	-11438.5
9	0.013	1.1	-8361.5

Table 4.7: concentration CO$_2$ measured during analysis of the air out of the syringe. The tap openend only in the direction of the syringe

Subsequently, measurements in the syringe were performed after filling the syringe with a 5 % CO$_2$ concentration. The results are shown in table 4.8.

Measurement	Real CO$_2$ concentration (%)	measured CO$_2$ concentration (%)	Deviation (%)
1	5	4	20
2	5	4.1	18

Table 4.8: CO$_2$ concentration measured during opening the tap only in the direction of the syringe. The syringe was filled with 5% CO$_2$.

Second method: Enlarge the volume of the cuff

Option 1, Enlarge the volume of the cuff

To enlarge the volume of the cuff, another endotracheal tube was used with a standard cuff of 7 ml.

CO_2 was measured after the following steps: 1. Let the Tonocap fill the cuff. 2. Empty the cuff with the syringe, register the amount of air. 3. Fill the cuff with the same amount of air with a CO2 percentage of 5%. 4. Analyse the air by the Tonocap.

The adjusted Tonocap was able to fill and analyse the content that was blown into the cuff.

Measurement	Real CO_2 concentration (%)	Measured CO_2 concentration (%)	Deviation (%)
1	5	3.3	34
2	5	3.1	38
3	5	3.8	24
4	5	3.7	26

Table 4.9: CO_2 concentration measured with the tap only opened in the direction of the normal endotracheal cuff. The cuff was filled with 5% CO_2 concentration.

The adjusted Tonocap is able to fill and to analyse the content that was blown into the cuff. Table 4.9 shows a CO_2 percentage that is lower than the correct 5%. The average deviation is 30.5 %.

During step 3, the air in the syringe is also analysed by the CO_2 sensor. The outcome were: 4.6 % en 4.4%. These values are lower than the expected 5%.

4.2   Part 2: Gold standard

4.2.1   Step 1: First criteria

In the literature review, 39 possible techniques were found for measurement of the microcirculation (appendix 1). To shorten this list of potential techniques, for every technique a consideration is made whether it fits the requirements set in the list of criteria. The evaluation of the 39 techniques are evaluated started with the first criteria applicability. When the technique seemed applicable evaluation based on the other criteria followed. When the technique did not fit one of this criteria, it was excluded as a potential gold standard. After this first evaluation, six techniques remained feasible as a gold standard because these were the only techniques that met all of the previously stated criteria. The remaining techniques are: Pulsed Wave High Frequency Doppler (PW HFD), Laser Doppler Flowmetry (LDF), Near Infrared Spectroscopy (NIRS), Photopulse Plethysmography (PPG), Sidestream Dark Field (SDF) and Scanning Laser Doppler (SLD).

	PW HFD	LDF	NIRS	PPG	SDF	SLD
Applicability	yes	yes	yes	yes	yes	yes
Validation	yes [15]	yes [70]	yes [71]	yes [29]	yes [36]	yes [10]
Size	0.8 - 2 mm tip size [55]	11 x 20 mm [63]	10 x 30 mm [58]	16 x 17 mm [8]	40 mm diameter [66]	10 x 30 mm [58]
Depth	4.0 mm [15]	2.0 mm [70]	2.5 cm [45]	2 mm [11]	3 mm [3]	0.3 mm [40]
Continue	yes	yes	yes	yes	yes	yes

Table 4.10: The six techniques that met all of the stated criteria: aplicability, validation, size, depth of measurement and continue measurements.

4.2.2 Step 2: Evaluation of the remaining techniques

The six techniques that remained feasible as a possible gold standard after the first evaluating were examined further based on the stated questions. The results are showed in the table below.

	PW HFD	LDF	NIRS	PPG	SDF	SLD
1	−	++	−	−	+	−
2	0	++	0	−	++	−−
3	0	+	−	−	+	0
4	−−	++	+	−−	+	+
5	−	++	−	−−	+	−

Table 4.11: Overview of the evaluation of the six left over techniques based on the score on the next five points. 1: Is the technique mentioned as a gold standard in literature?, 2: For which applications is the technique suitable?, 3: Does the technique need adjustments for usage in the trachea?, 4: Is the technique still in use and is it udes for a longer period?, 5: Is the technique likely to be functional for measuring microcirculation in the tracheal wall?. ++: excellent, +: good, 0: sufficient , −: insufficient, −−: poor

Starting with PW HFD, it has not been mentioned as a gold standard. The technique has been used frequently. However it does not seem to be used much anymore for measuring microcirculation. Most publications predate 1997.[6] Moreover this technique has not been tested on human tissues before 1997.[15]

LDF has been used in many applications and is used as a gold standard to validate other techniques.[44]
citeMerla2008citeMoorInstruments2007 The technique has been developed in 1972 [70] and is still in use. Therefore LDF is likely to be functional for measuring in the tracheal wall.

NIRS is not stated as a gold standard and has mostly been used in the cerebra and muscles. [28][65] NIRS needs many adjustments to make it suitable for measuring in the trachea.[65][82] NIRS is developed in 1977 and is still in use.[57] Therefore this technique is not likely to be applicable in the trachea.

32

PPG is not mentioned as gold standard. It is mainly used to measure fingertip skin blood flow.[21] Besides this technique is used since the 1930's [46] and has not been used freqeuntly on measuring microcirculation specifically.[39]

SDF has been used as gold standard to validate several other techniques[2][7] and is validated for several tissues [29][36][77][79] The technique is used for many publications [73]. No adjustments are needed for application in the trachea. The technique has been developped in 2005 [36]. SDF is likely to be functional for measuring microcirculation in the tracheal wall.

SLD is not mentioned as a gold standard in literature and has only been used to measure retinal microcirculation.[51][34][40] The technique was developed in 1991 and is still is in use.[23] Therefore this technique is not likely to be functional for measuring microcirculation in the tracheal wall.

4.2.3   Step 3: Recommended techniques

The two remaining techniques for the gold standard are LDF and SDF. LDF is an accurate and reliable method for assessing microcirculatory function.[70] Besides, LDF has been stated as a gold standard in literature for measuring microcirculation.[48][56] SDF is an accurate method to measure microcirculation and has been used to validate near-infrared laser speckle imaging [7] and the Cytocam-IDF imaging device [2].

Laser Doppler Flowmetry

LDF is based on the Doppler effect. The Doppler effect is based on light that is scattered by a moving object that undergoes a Doppler shift. The gratitude of this frequency shift depends on the movement of the object, the direction of the incoming light and the direction of the scattered light. In biological tissue photons are multiple scattered, because of the turbidity of the tissue. However, these scattered beams will not undergo a noticeable frequency shift when the tissue does not move. Therefore the flowing red blood cells (RBCs) will mainly cause the noticeable frequency shifts.[26] During LDF, a beam of two milliWatt He-Ne laser light is directed by an optical fiber to the probe head (Figure 4.5). The laser beam can penetrate to 1.5 to 2 millimeters depth. All RBCs traversing this volume will partly reflect this laser beam. Therefore the laser beam will undergo a frequency shift and the beam will consist of Doppler-shifted and unshifted components. The movement and number of RBCs will define the magnitude and frequency distribution of the Doppler-shifted component.[70] The backscattered light will be picked up by two optical fibers which direct the signal to photodetectors. The photodetectors then convert this light into electrical signals with the same frequencies as the backscattered light. Thereafter the difference between the both components can be determined.[70]

The output value for LDF is therefore not an absolute measurement of flow, but is proportional to the mean velocity and concentration of red blood cells in the circulation.[35][42] However these measured values are not absolute but relative, they are comparable

at the same time and in the same object. The measured values can also be correlated to values measured at the same time from another point of the investigated tissue.[42]
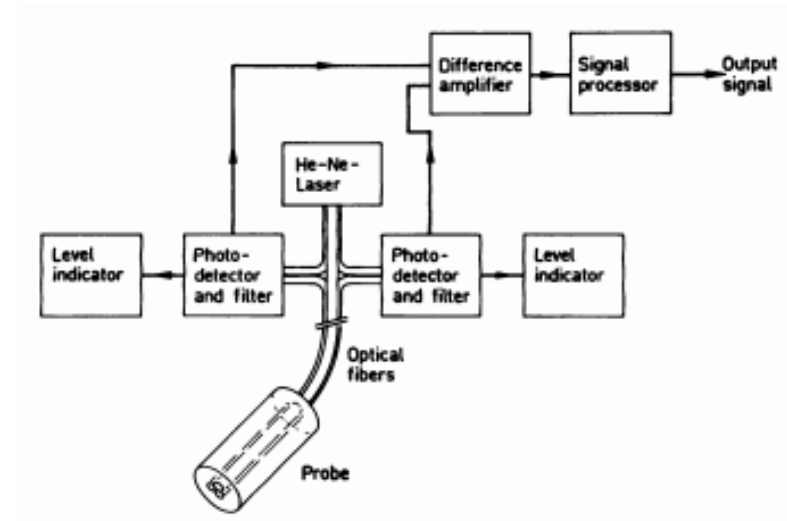

Figure 4.5: A simplified schematic of a 2 channel LDF system with the He-Ne laser source, the probe with optical fibers and the photodetectors.[70]

Sidestream Dark Field

The principle of Sidestream Dark Field arises from dark field microscopy. In a dark-field microscope, objects are illuminated against a dark background. To accomplish this, a special kind of condenser is needed. This condenser has a dark-field stop. This is an opaque disc that obstructs the path of light from the central light source, but allows penetration of peripheral light. A hollow cone of light is transmitted from the peripheral light. This hollow cone converges on the object and diverges from there as an inverted cone. When objects are present, light rays can be scattered by them. These diffraction rays enter into the objective. Thus, the objective appears bright in a dark microscopic field.[52][54]

SDF imaging uses a method in which light-emitting diodes (LEDs) are used that emit 540 ± 50 nm (green light). These LEDs are arranged in a ring around the tip of the probe. (Figure 4.6) This light is absorbed by hemoglobin (Hb) in erythrocytes. By absorbing the light, the erythrocytes appear as clear dark bodies moving through the microcirculation. This provides sidestream dark field illumination. Light from the illuminating outer core of an SDF probe penetrates the tissue and illuminates the tissue-embedded microcirculation by scattering.[29] Also the illuminating light source is optically isolated from the emission light path in the core of the light guide. In this way light can penetrate more deeply, illuminating the microcirculation from the interior.The probe of the device is covered by a disposable cap. This disposable cap is not in direct contact with the

tissue which means that no direct surface reflections can interfere with the signal.[36]
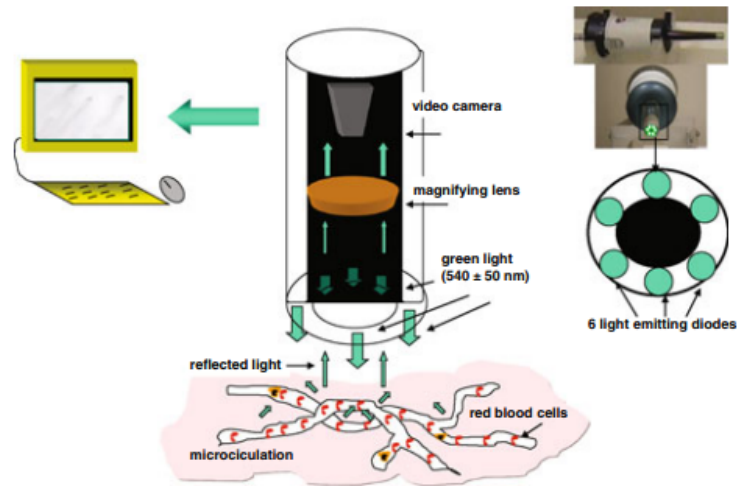
Figure 4.6: SDF (Sidestream Dar Field imaging) orthogonal reflected light goes through a dark path and becomes captured by the videa camera. The SDF makes an image of the microcirculation. [76]

SDF is able to produce a clear image of the microcirculation and its components (Figure 4.7). Erythrocytes and leukocytes can be observed with high resolution and deep monitoring capabilities. Image improvement is achieved by synchronising LED illumination with the video frame rate. Images output of the SDF devices show RBCs flowing as dark moving globules against a white or grey background.[31]
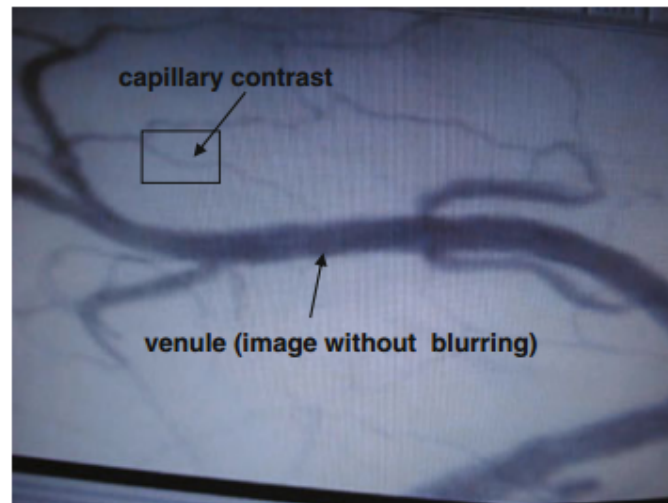
Figure 4.7: Microcirculatory images generated by SDF imaging in the sublingual mucosa[76]

4.2.4   Step 4: Recommended devices

From these two techniques three devices, which use these techniques, appear recommendable. Namely, the O2C device, the Microscan and the Cytocam. In the O2C a combination of LDF and tissue spectroscopy is used. The Microscan uses SDF and the Cytocam uses a successor of SDF which is called Incident Dark Field (IDF).

Microscan

Image output of the Microscan shows RBCs flowing as dark moving globules against a white or grey background. The probe of the device is covered by a disposable cap without being in direct contact with the tissue. This means that no direct surface reflections can interfere with the signal.[36] Synchronising the LED light pulse illumination with the CCD camera frame rate resolves motion-induced haemodynamic blurring.[53]

Advantages

- The first validation of the Microscan was in 2007.[29]

- It uses validated automated analysis software to measure the microcirculatory parameters total vessel density (TVD), perfused vessel density (PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI) and heterogeneity index (HI).[77]

- The technique uses a probe small enough to measure sublingually. Therefore it is likely that the probe is small enough to measure together with the Tonocap in the trachea. The size of the probe can be seen in figure 4.8.

Figure 4.8: Microscan probe covered with a disposable cap. [73]

- According to Jean-Marc ter Riet, Co-founder and chief executive officer of Microvision Medical [personal communication, 1-6-2016] it is possible to measure microcirculation in the tracheal wall.

- The SDF technique used in the Microscan is used in over 200 publications.[73]

- Because LEDs are being used in the Microscan energy supply from a battery is sufficient. This makes the device portable.[36]

Disadvantages

- The Microscan has only been used in three applications and not in the tracheal wall. Namely in measuring sublingual microcirculation [36], cutaneous microcirculation [77] and measuring thickness of the vaginal wall [79].

- Pressure artifacts can negatively influence the image quality.[29]

Cytocam

After Ortogonal Polarization Spectral imaging (OPS) and SDF, IDF-imaging (a successor of SDF) can be seen as a third generation handheld microscope. The Cytocam (Braedius Medical, Huizen, The Netherlands) is based on IDF imaging.[2][27][77] The working principle of IDF is the same as that of SDF, except that SDF separates the incoming light from the reflected light and IDF illuminates the field in a non-homogeneous way.[77] The Cytocam consists of a pen-like probe. This probe incorporates IDF illumination with a set of high-resolution lenses. It therefore projects images on a computer controlled high-density image sensor that is synchronized to an illumination unit.[2]

Advantages

- It uses automated analysis software to measure the microcirculatory parameters total vessel density (TVD), perfused capillary density (PCD), perfused vessel density (PVD) and flow velocity.[50]

- The technique uses a probe small enough to measure sublingually. Therefore it is likely that the probe is small enough to measure together with the Tonocap in the trachea.The size of the probe is about the size of big pencil, as visible in figure 4.9. [50]

Figure 4.9: The Cytocam [50]

- According to Annette Veening, Marketing and Sales Manager and Clinical Study Coordinator at Braedius [personal communication, 1-6-2016] it is possible to measure microcirculation in the tracheal wall.

- The device has a low weight, high optical resolution and a large field of view.[77]

- The low weight of the device minimizes pressure artifacts.[77]

Disadvantages

- The Cytocam has not been used to measure microcirculation in the tracheal wall.

- The device is not wireless.

- The first validation of the Cytocam was in 2015.[2]

O2C-device

The micro-lightguide spectrophotometer O2C (Oxygen to See; LEA Medizintechnik, Giessen, Germany) uses a combination of LDF and tissue spectroscopy. The O2C transmits continuous laser light (830 nm and 30 mW) and white light (20 W, 500-800 nm, 1-nm resolution) into the tissue where it is scattered.[4][24][37]

The O2C uses white light to determine the oxygen saturation SO_2 and relative amount of hemoglobin (rHb). The amount of absorbed light by the tissue provides the tissue hemoglobin value. Hemoglobin is the strongest light absorber in tissue. Therefore this measurement represents a hemoglobin concentration per tissue volume. The outcome of the measurement is independent from the vessel density, vessel lumen and hemoglobin quantity in the blood.[4][18][24] The oxygen saturation of hemoglobin can be determined by using the changing color of the reflected light caused by a wavelength-dependent absorption.[24][37]

Similarly with the white light, laser light is used for calculating the relative blood flow.[37] The blood flow velocity can be determined by detecting, analysing and displaying the Doppler shift caused by moving erythrocytes. Also the number of moving erythrocytes can be defined, where after the number of moving erythrocytes times velocity is used to calculate relative blood flow.[4][18]

Because the O2C uses laser light and white light it can determine blood flow as well as oxygen saturation and the amount of hemoglobin.[18]

Advantages

- O2C has a broad field of application including organ transplantation, skin microvasculature, wound healing, diabetic foot, fap transplantation and hyperbaric medicine.[18] Therefore, it is likely the O2C will function in the trachea as well.

- O2C can measure relative blood flow, oxygen saturation and the amount of hemoglobin simultaneously.[18]

- The technique can use a microprobe with a diameter of only 2.6 mm.[43] Therefore it is likely that the probe is small enough to measure together with the Tonocap in the trachea. (Figure 4.10)

- Laser Doppler Flowmetry is stated as gold standard and O2C uses this technique next to tissue spectroscopy.[48]

- The O2C has been validated several times.[4][37]

Figure 4.10: The O2C probe, microprobe with a diameter of 2.6 mm compared with the size of a match. [18]

- According to Thomas Derfuss, Head management at LEA Medizintechnik [personal communication, 3-6-2016] it is possible to measure microcirculation in the tracheal wall.

Disadvantages

- The O2C has not been used to measure microcirculation in the tracheal wall.

- The device is not portable.

4.3 Part 3: Application of the adjusted Tonocap and the gold standard for validation

In the future, the in vivo validation will be performed in a goat. A goat is chosen because its trachea is similar in size and shape to that of a human.[72] Also invasive measurement techniques are allowed to be used on the animal.

Before the measurement takes place, the test animal has to be endotracheally intubated. After this, the trachea of the animal will be intersected longitudinally and opened up through the skin above the cuff placement. It is important that the microcirculation of the trachea remains intact. This incision with a length of 6 cm will be made at the medial line on the ventral side of the trachea, because of the blood supply. The length of 6 cm is chosen because the trachea has a total length of 11 cm [74] and the cuff takes up a space of 5 cm. Measurements will be performed directly on the tracheal wall and not via the throat. In that case, the tracheal wall is easy accessible and there is more space to measure. This makes it possible to measure simultaneously with the Tonocap and gold standard. This setup also ensures fast and efficient measurements. The probe of the O2C is as small as a match and will therefore fit next to the cuff in the trachea. The probe has to be placed perpendicular to the mucosa of the trachea.

The O2C gives values of blood flow, rHb and SO_2. This has to be compared with the outcome of the adjusted Tonocap, which is CO_2 concentration. To be able to compare these outcomes, measurements should be performed with different cuff pressures. These different cuff pressures correspond with different values of perfusion of the tracheal wall. During measuring with different cuff pressures it should be researched whether both techniques (O2C and the adjusted Tonocap) respond similar to a variation in perfusion.

Chapter 5: Discussion

5.1 Part 1: Measurements of the Tonocap

5.1.1 Step 1: Measurements in an environment with a constant CO_2 concentration

This study shows a constant absolute difference in outcome of the incubator minus the adjusted Tonocap of 0.5% and the CO_2 sensor minus the adjusted Tonocap of 0.4%.

The absolute deviation of 0.5% can be explained from the literature. It has been shown that the Tonocap underestimates the calculated CO_2 by an average of 10%. In the measurements, the relative deviation was exactly 10%.[30]

5.1.2 Step 2: Evaluation of the air tightness of the box

In the first measurement, there was a large absolute deviation of -6.2% which corresponds with a relative deviation of 20.1%. Therefore, the box was considered as not being airtight. For this reason the box has been adjusted by sealing the input/outputs.

The improvement of the box seemed successful. During measurement two, the CO_2 concentration is constant during the first 10 minutes. The concentration of CO_2 after 15 minutes measuring is remarkably high, which can possibly be caused by the release of the reaming CO_2, present in the CO_2 supply. For this reason, the CO_2 supply was blocked after supplying CO_2 during measurement three and four. Measurement three and four both showed a very small deviation of respectively +0.2% and -0.3%. The very small decrease in CO_2 concentration could be caused by the CO_2 sensor itself. The sensor sucks air from the box and creates an underpressure. As a result, room air enters the box and the CO_2 concentration in the box decreases, which explains the decrease of the CO_2 concentration. Another explanation for the decrease in CO_2 concentration is that the box is not entirely air tight. The self-made box is not fabricated professionally and has several ways to leak air. A third possible explanation is that the box can be permeable to CO_2. The increasing value of 8.5% and 5.9% during the 15-minutes measurement was unexpected, because the CO_2 supply was blocked. Possibly, the adjusted Tonocap measured a lower CO_2 concentration in the beginning of the test. A declaration could be that CO_2 was at the bottom of the box, because it has a greater mass that air. [68] After 10 minutes, more air will be disappear in comparison to CO_2 and the CO_2 concentration will increase.

5.1.3 Step 3: Measurements in an environment with a variable CO_2 concentration

The results showed a few invalid measurements (1, 2, 8, 9, 10). During the first invalid measurement, the adjusted cuff was positioned right under the CO_2 input, while the CO_2 sensor was positioned at the other side of the box. This can declare a higher CO_2 concentration measured by the adjusted Tonocap than by the CO_2 sensor. Furthermore, the adjusted Tonocap is positioned lower than CO_2 sensor and might therefore measure a higher CO_2 concentration. Because the CO_2 is mainly present at the bottom of the box.

During the second invalid measurement, two notifications showed up on the screen of the Tonocap: 'Catheter pressure dropped' and 'Catheter pressure under minimum'. Probably, there was a leakage in the system. As a consequence, the adjusted Tonocap might have measured a very low CO_2 concentration. Measurement eight and nine are not valid because the three measurements of the CO_2 sensor differed more than 3%. During measurement eight to ten, the CO_2 concentration was increased during measuring. This was possibly due to leakage of the CO_2 via the CO_2 supply. After measurement ten, it was checked whether the input was still blocked and afterwards, the deviation of the CO_2 sensor was in a normal range.

Figure 5.1 shows that the CO_2 concentrations were significantly below the trend line, which has been described before and is also demonstrated by the paired T-Test. Interestingly, the study showed, substantiated by the coefficient of deviation, no linkage between the concentration of CO_2 and the relative deviation of the adjusted Tonocap. This means that the deviation of the adjusted Tonocap does not change with a change of CO_2 concentration.

Table 4.3 shows a maximum deviation of 10.8% between the adjusted Tonocap and the CO_2 sensor. It is assumed that the measured value by the CO_2 sensor is the real CO_2 concentration. However, step 1 showed that there is a deviation between the CO_2 sensor and the incubator of 2.7%. This means that the deviation between the adjusted Tonocap and the realistic CO_2 concentration is larger than step 3 shows.

5.1.4 Step 4: Adjustments of the cuff pressure

First method: Reduce the amount of gas

Option 1, reduce the amount of gas
When the pressure is reduced, the adjusted Tonocap measures low values of CO_2. The measured value differs for each measurement even when the pressure stays the same. This can be seen in measurements one and two and in measurements four and five. When the pressure in reduced to 0.03 bar, which would be an acceptable pressure for the cuff in the trachea, analysis of the gas does not give realistic values of the CO_2 concentration and the Tonocap gives the alarm 'pressure drops under minimum'. The Tonocap does not give this alarm when te path tot the venting system is open during

analysis. However, the pressure in the whole system remains the same. The absence of the alarm can thus not be explained using this argument. An explanation could be that the Tonocap needs more volume to make analysis of air possible. The fourth measurement during this experiment could be an example of this phenomenon. The outcome of this measurement has a more realistic value of CO_2. This may have occurred because the venting system was not completely closed. Therefore, analysis by the Tonocap was possible.

Option 2, reduce the amount of gas
The pressure regulator did not work in the set-up, because the cuff did not fill. The reason for this is probably the low amount of gas the Tonocap inflated. The amount of gas (5-8ml) injected by the Tonocap has a flow between 160-230ml/min, which will be pumped in in maximal three seconds.[75] This period is too short to work with the pressure regulator. Though the cuff would fill, it is still doubtful whether this technique works. The regulator cuts off the pressure at 0.03 bar. This does not implicate that the cuff will be inflated with this pressure. Probably more air is needed to reach a cuff pressure of 0.03 bar. Also the pressure regulator was not accurate enough for this measurements, it regulates pressure flow from 0-5 bar. Regulation of a pressure of 0.03 bar is difficult to establish with this regulator.

Option 3, reduce the amount of gas
The relative deviation between the expected CO_2 and the measured CO_2 by the adjusted Tonocap is very large. To investigate the cause of this problem, analysis of the gas from both sides (the syringe and the cuff) was done separately. Firstly, CO_2 concentrations measured in the cuff showed a larger deviation from the expected CO_2 concentration than measured before (step 1, measuring in an environment with constant CO2 concentration). There is no valid explanation for this difference except the low accuracy of the Tonocap.

Secondly, the gas from the syringe is analysed. The expected value of 0.013% CO_2 is in the measurement range of the Tonocap. In fact the Tonocap should be able to measure the value. However, the accuracy of 0.2vol% is not high enough. This could be an explanation for the different CO_2 values measured by the Tonocap. To minimize the large deviation between the measured CO_2 concentration by the sensor versus the adjusted Tonocap, the syringe was filled with 5% CO_2. Second analysis showed reduced deviations with a maximum of 20%.

Because the deviation of CO_2 concentration in the first and second part of the measurement the result was not of value, a conclusion could not be made.

Second method: Enlarge the volume of the cuff

Option 1, enlarge the volume of the cuff
Our study showed that the Tonocap is able to analyse the gas inside the cuff when a cuff with a higher volume is used. This volume enlargement might be a potential solution to lower the pressure in the cuff and still be able to measure the CO2 concentration. The

measured CO_2 concentration values from the cuff variate with every measurement. This can be explained by the usage of a normal endotracheal tube, which is not meant for CO2 measurements and is not permeable, but also not fully unpermeable for CO_2.

5.1.5   General points of discussion

Beside the points of discussion that were mentioned before, there are some general points of discussion which are applicable for the entire research. At first, the Tonocap is a very old device which has not been used for a long period. Therefore, the quality of the device is not optimal. For example; the volume that was injected into the cuff by the Tonocap was variable. This was evidently seen in the size of the cuff. When the device was turned off and put on again, this problem was resolved. This is remarkable, because the rest of the set-up did not change.

Next to this, the Tonocap very often showed the error 'pressure dropped'. It was expected that somewhere in the system was a leak, because the supplying system and taps did not fit well with each other and the Tonocap. Because of the absence of the right compounds, there is improvised to create a system which did not leak. The potential leaks could have led to measurement inaccuracies and may have made the measurements less reliable. Another point of discussion concerns the cuff that was used. This cuff derived from an original stomach tube and has therefore been stretched and touched many times. It is possible that the quality of the cuff and its CO_2 permeability has changed. This research could be improved by performing the measurement with multiple unused cuffs.

To prevent measurement inaccuracies, it was desirable to calibrate the Tonocap before the first measurements took place. However, the fluid to calibrate the Tonocap was not present. Therefore, the calibration could not be performed.

A general point of discussion, which is important to keep in mind when thinking about the pressure in the cuff, is the temperature. Following the equation of an ideal gas $p * V = n * R * T$, the temperature influences the pressure of the cuff. When the cuff fills with gas, the gas has the same temperature as the surrounding air in the room (293K). In the equilibration period of 10 minutes, the temperature will raise to the temperature of the surrounding tissue, which will be 330K. This will cause a gain in the pressure of the cuff .

Lastly, the instructions of the Tonocap were barely present. The manual was very technical and also Internet did not have more usable information. Because of this, it is not known whether all settings were set correctly and this might have influenced the results.

5.1.6   Processing of the data

Originally, the aim of this study was to analyse the data of the Tonocap using a connection between a serial- and an USB-gate, eventually in combination with Matlab. When the connection between the two gates was found, it became clear that a whole software program should have been written to analyse the data. This would have take

too much time for a bachelor thesis. Furthermore, the Tonocap showed clear CO_2 concentrations during the measurements and the software was not needed to analyse this value. Taking the time efficiency into account, it was decided to only use the showed CO_2 concentration value by the Tonocap.

5.2 Part 2: Gold standard

5.2.1 Step 1: First criteria

There were 39 techniques appropriate for measurement of the microcirculation found. It is presumable that there are more techniques available for measurement of the microcirculation than these 39 techniques. It is assumed that the most potential techniques for the gold standard are in this list. When a technique is not found after this big literature research it is probably not validated and applicable as gold standard.

The techniques were executed from the list when they did not meet one of the criteria. It is possible that a potential gold standard is missed, because it is executed from the list for one missing criterion. The information for the missing criterion might have been found in an unreliable source.

After this evaluation 13 techniques remained. These techniques were better checked with the same criteria. After this evaluation only six potential techniques were left. Because these six techniques stated all the criteria (Table 4.10) they are potential gold standards.

5.2.2 Step 2: Evaluation of the remaining techniques

Table 4.11 shows whether the six remaining techniques meet the questions set up in step two.

- PW HFD does not give an excellent or a good outcome for any of these questions which makes this technique not suitable as potential gold standard.

- LDF gives an excellent outcome for all the questions except for question three. It is expected that the technique does not need an adjustment for usage in the trachea. Because it is never used in the trachea this is not completely certain, hence it has a good score instead of excellent. Therefore, this technique is a potential gold standard.

- NIRS gives a positive outcome for question four, but does not give a good or excellent outcome for the other questions. This makes this technique not suitable as gold standard.

- PPG gives no positive outcome at all. This technique is unsuitable as gold standard.

- SDF is a potential gold standard. It gives good or excellent outcomes for all questions. The outcome for question three is good for the same reason as it is for LDF.

- SLD only gives a good outcome for question four, the technique is still in use but not potential as gold standard for measurement in the trachea.

5.2.3 Step 3: Recommended techniques

Both LDF and SDF meet the previous stated requirements. However, LDF complies better with the requirements. LDF is an older technique and therefore used more often and in more different applications than SDF. Next to this LDF is stated as a gold standard whereas SDF has only been used to validate two other techniques. Based on these differences, the technique that suits the stated requirements best is LDF. That is why it is recommended to use the technique of LDF for measuring microcirculation in the tracheal wall.

The tracheal wall has not been investigated a lot so far. Therefore it is difficult to predict whether the chosen techniques will function in the trachea.

5.2.4 Step 4: Recommended devices

In view of this advice it is recommended to use the O2C device, because LDF is incorporated in this device together with tissue spectroscopy. This composition of the two techniques is beneficial because it combines advantages of both of them. This means that blood flow as well as oxygen saturation and hemoglobin amount can be determined simultaneously.

5.3 Part 3: Application of the adjusted Tonocap and the gold standard for validation

It is expected that the suggested set-up will not give any problems. Because of the microprobe the curvature of the trachea will not have any influence on the measurements.

The O2C can not be placed exactly on the cuff site, but will be placed as close to the cuff site as possible. It is possible that measurement errors will arise. However, it is expected that the perfusion this close to the cuff is comparable to the perfusion at the cuff site.

Once every ten minutes the Tonocap will measure the CO_2 concentration. Therefore the Tonocap empties the cuff and the cuff pressure will drop dramatically. Hereby ventilation of the test animal will not be as effective as desired during this period. However, it is expected that this will not have an effect on the test animal, because the cuff is only empty for a few seconds every ten minutes.

Blood flow measurements by the O2C can be influenced by movement, colours, light and pressure. Mistakes that should be prevented are movement artifacts by respira-

tion movements, the use of coloured disinfectant, the influence of external light and application of the probe with too much pressure.[18]

Chapter 6: Conclusions

6.1 Part 1: Measurements of the Tonocap

6.1.1 Step 1: Measurements in an environment with a constant CO_2 concentration

From the performed measurements can be concluded that the adjusted Tonocap is constant in measuring CO_2 percentages. But there is a relative deviation of around 10%. There can be concluded that this was a successful first step in validating the adjusted Tonocap, due to the fact that all measurements were constant. The adjusted Tonocap is able to measure well, but with a deviation.

6.1.2 Step 2: Evaluation of the air tightness of the box

The box is not 100% airtight, but it is considered to be airtight enough to do further experiments.

6.1.3 Step 3: Measurements in an environment with a variable CO_2 concentration

The adjusted Tonocap can measure CO_2 concentration reasonably accurate. The deviation with the CO_2 sensor is between 1.8% and 10.7% lower. In agreement with the literature, the Tonocap had an average deviation of 10 %. Thus, the adjusted Tonocap is capable of measuring different concentrations of CO_2 adequately.

The statistic analysis shows a significant difference between the adjusted Tonocap and the CO_2 sensor and the concentration is not corresponding to the relative deviation. In conclusion, the concentration of CO_2 does not influence the deviation.

6.1.4 Step 4: Adjustments of the cuff pressure

To make the adjusted Tonocap a potential technique for measuring the perfusion in the trachea, the pressure of the cuff needs to be reduced. There are two potential ways to regulate this pressure. The first one is reducing the amount of gas blown in the cuff. Concluding from the tried methods this is not a potential solution. The Tonocap does not measure accurate enough to give a valuable outcome. The second method is to use a cuff with a bigger volume. The Tonocap did not measure the right value with the used

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cuffs in the experiment. Nevertheless, this method has potential to become a solution in clinic. When a larger tonometry cuff is used it would be possible to measure the right CO_2 and give a lower cuff pressure.

6.2   Part 2: Gold standard

6.2.1   Step 1: First criteria

Six techniques remained feasible as gold standard after the first evaluation. These six techniques are: PW HFD, LDF, NIRS, PPG, SDF and SLD.

6.2.2   Step 2: Evaluation of the remaining techniques

LDF and SDF are likely to be functional as a gold standard to measure microcirculation in the tracheal wall.

6.2.3   Step 3: Recommended techniques

LDF is best suited as a gold standard.

6.2.4   Step 4: Recommended devices

O2C is recommended as a device to measure microcirculation in the tracheal wall.

6.3   Part 3: Application of the adjusted Tonocap and the gold standard for validation

The adjusted Tonocap can measure CO_2 concentrations sufficiently, but with a 0-10% deviation lower than the actual concentration. For that reason, in vivo experiments with the adjusted Tonocap can be performed to validate its usage. However, beforehand, the endotracheal cuff pressure should be lowered. This is impossible by reducing the amount of gas, but seems possible by enlarging the volume of the cuff.

LDF can be used as a gold standard to validate the adjusted Tonocap. The O2C device uses LDF in combination with tissue spectroscopy. This combination provides reliable measurements of the microcirculation in the tracheal wall. Therefore usage of the O2C is recommended for future validation of the adjusted Tonocap.

To realise the validation, part 1 and part 2 have to be combined. Measurements should be carried out with the adjusted Tonocap and O2C together in the trachea. It is likely that both techniques fit together in the trachea. The outcomes of the O2C and adjusted Tonocap should be compared during measurements with varying cuff pressures.

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Appendix

Appendix 1: List of 39 techniques

This appendix shows a list of the 39 techniques that could measure tissue perfusion.

- Blood gas analysis
- Capnography
- Color Doppler Ultrasonography
- Digital Substraction Angiography
- Dynamic Computed Tomography
- Electron Paramagnetic Resonance Spectroscopy
- Ibidi OPAL
- Indocyanine Green Video Angiography
- In Vivo Fluorescence Microscopy
- Intravital Microscopy
- Lactate Measurements
- Laser Doppler Flowmetry
- Laser Speckle Imaging
- Microelectrode Techniques
- Microdialysis
- Microspheres
- Mixed Venous Oxygen Saturation
- MRI with Arterial Spin Labeling
- Multiple Indicator Dilution Technique
- Near-infrared Spectroscopy
- Micro-lightguided Spectrophotometry
- Magnetic Resonance Spectroscopy
- Orthogonal Polarization Spectral Imaging
- PET/SPECT
- Pholarographic electrodes
- Photopulse Plethysmography
- Pulsoximetry
- Resonance Raman Spectroscopy of Hemoglobin
- Scanning Laser Doppler
- Side Stream Dark Field
- Second Harmonic Ultrasonic
- Blood Perfusion Measurement
- Thermal Diffusion Technology
- Three-dimensional Computed Tomography Angiography
- TOP-cam
- Tissue Oximetry
- Transcutaneous Oxygen Electrodes
- Two-slit Photometric Measurement Technique
- Ultrafast Computed Tomography
- White Light Spectroscopy