STENTING OF THE _TUBA AUDITIVA EUSTACHII_ AS A VALID TREATMENT CONCEPT IN CHRONIC OTITIS MEDIA, CHRONIC AUDITORY TUBE DYSFUNCTION AND ITS SEQUELAE:

IMPLEMENTATION OF A LARGE ANIMAL MODEL

THESIS

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Friederike Pohl
Hildesheim

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Supervisor: Prof. Prof. h.c. Dr. Thomas Lenarz

Supervision Group: Prof. Dr. Elke Zimmermann
Prof. Dr. Christian Hartmann
Prof. Dr. Hans Gerd Nothwang

1st Evaluation: Prof. Prof. h.c. Dr. Thomas Lenarz
Clinic for Laryngology, Rhinology and Otology
Hannover Medical School

Prof. Dr. Elke Zimmermann
Institute of Zoology
University of Veterinary Medicine Hannover, Foundation

Prof. Dr. Christian Hartmann
Institute for Pathology, Neuropathology
Hannover Medical School

2nd Evaluation: Prof. Dr. Holger Sudhoff
Clinic for Ear, Nose and Throat Clinic,
Head and Throat Surgery
Bielefeld Mitte Clinical Centre

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To

My Family and Friends

It matters not how strait the gate, how charged with punishment the scroll,

I am the master of my fate: I am the captain of my soul.

William Ernest Henley (1849-1903)
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<td>OME</td>
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<td>CSOM</td>
<td>Chronic suppurative otitis media</td>
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<td>COM</td>
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Summary

Stenting of the *Tuba Auditiva Eustachii* as a Valid Treatment Concept in Chronic Otitis Media, Chronic Auditory Tube Dysfunction and its Sequelae: Implementation of a Large Animal Model

Friederike Pohl

Acute and chronic otitis media are among the most common diseases in children and adults. Approximately 20% of acute otitis media persist longer than three months and turn into chronic otitis media. Eardrum perforation, ossicular chain disruption and other severe damages of the middle ear can be the result and hearing impairment as well as hearing loss the long term consequences. Thus, an early successful treatment, preventing chronic otitis media and severe hearing impairment, has to be one of the major goals in otorhinolaryngology. Despite profound improvements in the development of therapeutic options, few approaches deal with the Eustachian tube itself, which is one of the key factors in the development of these diseases. Valid therapeutic interventions to restore Eustachian tube function are not available hitherto. The minimal invasive implantation of stents into the auditory tube to improve middle ear ventilation and facilitate tubal opening could be an innovative option. One of the obstacles is a missing *in vivo* model where stents in their original dimension for human use can be tested pre-clinically. Thus, the aim of this thesis was the implementation of a minimal invasive surgical approach for the *in vivo* stenting of the Eustachian tube in a large animal model and in addition, to identify a method for the functional evaluation of the implanted medical device.

For the evaluation of the middle ear function tympanometry in conscious sheep was performed. Sheep were used as animal model based on previously performed cadaver studies identifying this species as the ideal candidate. The presented methods for the evaluation of a stent implanted in the Eustachian tube are the following: the minimal-invasive method of stent application, induced aseptic middle ear inflammation with effusion, evaluation of the *in vivo* reaction on the implant in the region of the pharyngeal orifice by means of an endoscopic score, tympanometry for the assessment of middle ear function and structures, computed tomography for the assessment of location of the stent and the ventilation of middle ear and stent, and histologic analysis of tissue formation, stent diameter and regeneration or reparation of prismatic and ciliated epithelium. Commercial coronary stents were bilaterally implanted into the Eustachian tubes of three blackface sheep and remained implanted for 12 weeks.
Implantation of the stent was possible in each case. The used combination of *in vivo* and *ex vivo* tests displayed ventilated middle ears and incorporation of the struts into tissue topped with ciliated epithelium. The stent was expanded in all 4 parts of the analysed section of the Eustachian tube. Additionally, the stent appeared to be sufficiently fixed in the cartilaginous part of the Eustachian tube. In endoscopic examination of the external auditory canal no symptoms of otitis externa, eardrum perforation or otitis media with effusion were apparent. Tympanometry provided reliable results for the evaluation of middle ear function in conscious sheep. Thus, it can be inferred that this combination of tests already provided a diverse spectrum of data, facilitating the evaluation of the studied stent. In general, the methods were very well suitable to provide the necessary data according to the used stent.

It can be concluded that the here presented assessment of stents in the large animal model of the sheep provide a profound basis for the testing of these kinds of medical devices in pre-clinical studies. The transfer of the animal model from *in vitro* to *in vivo* could be demonstrated, as well as the suitability of the proceeded tests for the valuation of the used implant.
Zusammenfassung

Stentimplantation in die *Tuba Auditiva Eustachii* als wirksames Behandlungskonzept der Chronischen Mittelohrentzündung, Chronischen Tubenfunktionsstörungen, sowie dessen Folgen: Einführung eines Großtiermodels

Friederike Pohl


In der vorliegenden These konnte gezeigt werden, dass die präsentierten Methoden zur Untersuchung eines Stents im Großtiermodell Schaf eine profunde Basis zur Testung dieser Art von Medizinprodukten in prä-klinischen Versuchen bieten. Der erfolgreiche Transfer von *in vitro* zu *in vivo*, wie auch die Eignung der verschiedenen Testverfahren für die Evaluierung der hier verwendeten Stents konnten ausreichend demonstriert werden.
1. General Introduction

Acute and chronic otitis media (AOM, COM) are among the most common diseases in children and adults. By their third birthday 80% of children suffered at least once from acute otitis media and by their seventh year of life 40% experienced six or more episodes \(^1\). But not only children are affected. The World Health Organisation estimated a prevalence of 65 to 330 million individuals suffering from chronic suppurative otitis media (CSOM), a complication of AOM. Additionally, 28 thousand deaths occur each year in the developing countries that can be associated with otitis media \(^2\). Approximately 20% of AOM persist longer than three months and turn into COM. Eardrum perforation, ossicular chain disruption and other severe damages of the middle ear are the result and hearing impairment as well as hearing loss are the long term consequences \(^1\). Despite improved diagnosis and treatment, still serious complications occur. Since hearing plays an important role in the development of language, impaired hearing at a young age may disturb proper language acquisition and social skills in the interaction with equally aged children. But also adults suffering from hearing impairment are affected in their social life. Thus, an early successful treatment, preventing COM and severe hearing impairment, has to be one of the major goals in otorhinolaryngology. AOM has two maxima in appearance: the age of two and five years \(^1\). Differences in the anatomy of the Eustachian tube (length of the tube and shape of the pharyngeal orifice) and efficiency of the muscles of the *tensor veli palatini* of the infant compared to the adult can be related to the increased incidence of middle ear disease in children \(^3\). Thus, one of the essential factors in the development of this disease is the dysfunctional Eustachian tube, hampering the pressure equilibration in the middle ear and the transport of secretion from the middle ear to the nasopharynx \(^4\). However, valid therapeutic interventions to restore Eustachian tube function are not available hitherto. Stenting of the Eustachian tube has been pursued in the past and several devices (see Chapter 1.1) with varying success have been developed for clinical use. One of the obstacles is a missing *in vivo* model where stents in their original dimension for human use can be tested pre-clinically. Thus, the aim of this thesis was the implementation of a minimal invasive surgical approach *for the in vivo* stenting of the Eustachian tube in a large animal model. For the evaluation of the middle ear functions, tympanometry in conscious sheep was performed. Sheep were used as animal model based on previously performed cadaver studies \(^5\) identifying this ruminant species as the ideal candidate. In the next chapters anatomy and physiology of external ear, middle ear and Eustachian tube are described to illustrate the functional unity of this system. In addition, the method of tympanometry for the assessment of middle ear structures and auditory tube function
as well as the animal model of the sheep for pre-clinical studies, testing middle ear and Eustachian tube implants are introduced briefly.

1.1 Historical Considerations of the Eustachian Tube and Stenting as a Treatment Option for Specific Manifestations of Otitis Media

The idea that ear and nose are connected is much older than the first description of the anatomy of the Eustachian tube. Already in the antiquity in the “Problemata Physica” associated with the “Corpus Aristotelicum”, the reflex of coughing when the patient is scratched in the outer ear canal was attributed to the fact that ear and nose are connected. However, the anatomy of this tubal connection between ear and nose was firstly documented by Bartholomeus Eustachius in 1563 in Rome, postulating that the knowledge of this tube will be helpful for physicians to improve the use of therapeutics. The Eustachian tube has various names, referring to supposed function: auditory tube, the discovering scientist: Eustachian tube or the anatomy: tuba pharyngotympanica. It can as well be found in many mammalian species and is schematically depicted in Figure 1.1 for the sheep.

The following passage is summarised from the textbook “Images from the History of Otorhinolaryngology”, presenting important findings in history of otorhinolaryngology. Based on Bartholomeus Eustachius findings, the “Masticatoria”, chewable viscous aromatic substances, became fashionable to stimulate saliva production. Drainage of secretion and pus from the middle ear should be the result. The therapeutic approach of chewing and therefore swallowing is still used today in airlines to facilitate pressure equalisation between middle ear and nasal cavities during take-off and landing of the plane. Approximately 100 years later, influential physical discoveries in regard to pressure, volume and temperature like the first mercury barometer of Pascal in 1648 or the Boyle-Mariotte law of gases in 1661/62 consolidated Joseph Duverney (1648-1730) and Antonio Maria Valsalva (1666-1723) in the idea that the renewal of air in the tympanic cavity might also be an important function of the Eustachian tube. This was considered as essential to maintain the elasticity of the “sensible parts” of the middle ear.
Duverney believed that the warm blood in the middle ear induces air expansion and generates pressure, which would be compensated by the auditory tube. Hence, the tube needed to be permanently open to prevent a withdrawal of the eardrum and therefore sustains hearing abilities. In contrast to this, Valsalva, who identified muscles for the opening and closing of the Eustachian tube, believed that the tube opens automatically during the process of hearing to improve the movement of the eardrum and that with enforced exhalation with nose and mouth closed the retracted eardrum could be popped into its original position. The Valsalva manoeuvre was born. Accompanied with the discovery of the tubal anatomy went the application of substances or rinsing fluid into the middle ear via catheters through the Eustachian tube. Guyot, originally a postmaster with diseased ears himself, invented in 1724 a double pump to rinse the auditory tube through the mouth. Archibald Cleland, however, was the first to approach the auditory tube via the nose. Several differently curved catheters should secure the blind approach of the nasopharyngeal orifice. Jonathan Wathen provided the first anatomic correct drawing of
the nasal approach in 1756. Now, the catheterisation of the Eustachian tube was suitable for the mass. After several severe complications resulting in death in the 19th century, the enthusiasm subsided and physicians became more carefully. In 1853, Joseph Toynbee presented a method for the functional testing of the Eustachian tube, by using the Valsalva manoeuvre in combination with a single act of swallowing. With a flexible tube from the ear of the patient to his own ear, Toynbee could hear a sound in the so called “otoscope”. Adam Politzer invented in the middle of the 19th century the “Politzer balloon”, which was used to inflate the Eustachian tube and therefore the middle ear. By this time, the consent was that some diseases of the middle ear could be cured by the restoration of adequate middle ear ventilation and that irritating substances or gas applied via a tubal catheter did have a negative effect. For approximately 100 years the Politzer balloon was the most common therapy for diseases of the ear until the ventilation tube or “grommet” in the eardrum replaced it. However, Toynbee and Valsalva manoeuvre are used as diagnostic tools until today.

One of the first documented attempts of implanting wires, tubes or stents into the Eustachian tube can be found in 1955, when Fritz Zöllner tried to improve Eustachian tube function by a polyvinylchloride tube with an attached thread inserted via the perforated tympanic membrane. The thread remained in the tube for up to 10 days. Complications like eardrum perforation or granulation tissue reduced the success. In 1977, Wright Jr. & Wright developed an Eustachian tube prosthesis, the so called Silastic tube, inserted via the tympanic orifice of the Eustachian tube for a longer period. Nonetheless, Lesinski reported in 1980 that the tube did not provide the expected long lasting improvement of Eustachian tube function. Based on these findings, Steinbach developed a gold wire, formed as a channel: the tube conductor, staying in the Eustachian tube for years with rare rejection and initially good functions. In the follow-up, only 8.3% of patients could benefit from this treatment. In 18.4%, the stent even needed to be surgically removed due to dislocation or formation of granulation tissue around the tube conductor. Another approach was performed in the chinchilla using a poly-L-lactide Eustachian tube stent with moderate outcome. Application was performed via the bulla, a part of the tympanic cavity, but due to oversize of the utilized stent, transient otorrhea and mild inflammatory response were observed. In 2011, the same group assessed this stent, which was sectioned lengthwise and implanted into middle ear and Eustachian tube of rabbits, producing otitis media in 50% of the animals until the 12th week after implantation. The insertion of the various ET implants was hitherto performed via the tympanic membrane or the tympanic bulla. A recent cadaver study in the sheep proved the possibility of endoscopic insertion of a commercial coronary stent through the nasopharyngeal orifice of the Eustachian tube to be
1. GENERAL INTRODUCTION

successful. In contrast to the blinded catheterisation, the insertion was performed under visual control via an endoscope to prevent complications. Despite of many approaches and materials in history, no effective treatment option addressing the Eustachian tube could be found. And furthermore, even the functions of this tubal structure still riddle physicians and scientists.

1.2 Anatomy and Physiology of the Ear

The ear can be classified into three parts according to their location and functions: the outer or external ear, the middle ear and the inner ear (Figure 1.1).

1.2.1 The External, Middle and Inner Ear

The external ear consists of the pinna and the external auditory canal (EAC). The pinna is located on the left and right side of the head and captures the sound from the surrounding, transferring the acoustic vibration into the EAC onto the eardrum and via the middle ear (ME) to the oval window of the inner ear. Because of its elastic cartilaginous formation not only conduction of the sound into the funnel of the EAC and therefore intensity enhancement is provided by the pinna but also spectral modulation of the sound frequency. Based on the spectral modulation and the bilateral location on the head, the pinna delivers important cues for the ability of spatial hearing. In humans, the EAC measures up to 3.5 cm merging from a cartilaginous elastic part into a bony part finishing with the eardrum, which divides the EAC from the ME. The isthmus, a narrowing of the EAC with a marginal curvature, marks the transition of these two parts. The EAC shows the typical dominant resonance of a tube that is closed on only one end causing molecule deflection decreases and pressure increases in direction of the closed end. These phenomena improve the power transfer onto the tympanic membrane in some species up to 100%.

The middle ear is composed of the air filled cavum tympani and the cellulae mastoideae, the tympanic membrane and the ossicular chain. The ET, however, is also defined as structure of the middle ear, but will be discussed in the next subchapter. The middle ear is covered with goblet cells and prismatic ciliated epithelium of various manifestations alike the nasal cavities. The cavum tympani can be divided into three merging levels: the epitympanon or atticus, the mesotympanon and the hypotympanon including the protympanon describing the area around the tympanic orifice of the ET. The eardrum, separating the EAC from the middle ear, consists
of a pars tensa and a pars flaccida with the “spine of the eardrum”, the manubrium mallei, connecting it to the ossicular chain. The cavum tympani is the location of the ossicular chain being composed of three tiny bones: the malleus, the incus and the stapes. It is held in position by two small muscles: the musculus (M.) stapedius and the M. tensor tympani. Via this formation of bones, muscles and tendons, the tympanic membrane is linked to the oval window. The most important physiological function of the middle ear is the transmission of the sound from the air phase of the external ear to the fluid phase of the inner ear, matching the impedance of these two different media. The approaching vibration is transferred and concentrated from the large low-impedance tympanic membrane to the small high-impedance oval window, hampering the reflection of the sound and enhancing its energy. Additionally, the lever action of the ossicular bones due to their different size (smaller incus arm and larger malleus arm) increases the force at the stapes. The cavum tympani gives the structures of the eardrum and the ossicular chain enough space to vibrate in its air filled cavity and therefore couples tympanic membrane and cochlear best.

The inner ear is embedded in the depth of the temporal bone and formed by the labyrinth and the cochlea. Three semicircular canals according to the three spatial planes form the labyrinth for the sense of balance. The cochlea is constructed of a coiled basilar membrane and three longitudinally divided scalae. These structures spiral around the axis of the modiolus from the base to the apex. Scala vestibuli and scala tympani are filled with perilymph, whereas the scala media is filled with endolymph. The inner and outer hair cells are located on the basilar membrane in the organ of Corti. Exactly positioned for the tonotopic acquisition of sound, they conduct the stimulus via the afferent fibres of the auditory nerve into the auditory pathway.

The anatomy and functioning of the inner ear is not relevant for this thesis and will therefore not be described in detail.

1.2.2 The Tuba Auditiva Eustachii

The Eustachian tube or auditory tube presents the only connection from the middle ear to the external surrounding e.g. the nasal cavities. It is shaped like two cones meeting at their tip. One cone consist of an inelastic bony part, beginning in the protympanon of the middle ear and covering one third of the total length (approximately 3 cm) of the ET. It merges into the other cone, an elastic cartilaginous part covering the additional two third of the ET ending in the nasopharynx. The conjunction of the cone tips creates a narrow passage called isthmus and measures up to 1.8 mm in length. The auditory tube starts in the nasopharynx lateral from the
pharyngeal space and proceeds in latero-superior direction to the middle ear. It forms a 45° angle to the axis of the sagittal frontal and horizontal plane of the skull. The cartilaginous part is located directly under the skull base lateral from the pharyngeal space. In contrast to other fluid transporting structures of the body like vessels, the ET is more formed like a gap than a tube. The cross section of the cartilage (Figure 1.2) looks like a hook or a shepherd’s crook but strongly varies in appearance as MRI studies proofed.

The elasticity of this cartilage is comparable to those of the pinna or the nose. Four muscles surround the ET inducing its active opening during swallowing or yawning. The M. levator veli palatini origins at the petrous part of the temporal bone and has its onset in the soft palate. Although it is one of the most important muscles of the soft palate, its impact on the ET may be marginal and results in compressive influence. The M. tensor veli palatini is the most important muscle for tubal functions. It origins in the scaphoid fossa of the skull base and the lateral cartilage of ET and forms a triangle to the processus pterygoideus. It is said to open the cranial part of ET pulling the lateral lamina of the medial wall. The Ostmann’s fat pad located on the medial mobile part of ET is pressed to the lower portion of the ET by this muscle and causes a compression and therefore movement of secretion in direction of the nasal cavities. The
palatopharyngeal muscle and the salpingopharyngeal muscle form a functional unit and elevate the pharynx: they may work as an anchor chain keeping the pharyngeal orifice in position. The medial pterygoid muscle works as a hypomochlion for the tensor veli palatini: by closing of the mouth, this chewing muscle contracts pushing the tensor in direction of the ET. Therefore, a contraction increases, whereas relaxation decreases the opening pressure of the ET. However, the total effect of each specific muscle is still not fully understood.

The ET is secured in its position by a medial and lateral suspensory ligament aligning the small medial Ostmann’s fat pad. The cross sectional view of the lumen of the auditory tube has already been described by Rüdinger in 1870 (Figure 1.2). The cranial portion of the lumen between lateral and medial lamina of the cartilage is called Rüdinger’s safety canal and appears to be always open, warranting the ventilation of the middle ear. Contrary to this, the auxiliary gap, e.g., the space below the safety canal only opens passively for the equalisation of high pressure in the middle ear or for clearance and protects the ET from pathogenic germs ascending from the nasal cavities. Additionally, MALT-cells, located in longitudinal mucosa folds along the ET and the pharyngeal tonsil at the pharyngeal opening, provide immunologic response. Over the total length of ET, ciliated epithelium occurs being more prominent in the nasopharyngeal portion and decreasing to small cilia on prismatic cells in direction of the middle ear. The goblet cells in the mucosal layer constantly produce mucus, keeping the ET moistured and helping to transport pathologic germs back to the nasal cavities. The most important functions of the ET are therefore transport of secretion, ventilation of the middle ear and protection against pathologic germs (Figure 1.3).
1.3 Pathophysiology of Otitis Media and Eustachian Tube Functions

Otitis media (OM) is defined as an inflammation of the middle ear, especially the tympanic cavity with several manifestations: Acute otitis media (AOM) has an acute beginning and is short termed in its duration. Otitis media with effusion (OME) is defined as an accumulation of non-purulent fluid behind the non-inflamed and intact eardrum being either serous or mucoid. It is referred to as glue ear if the fluid is thick. If these symptoms persist longer than three months and inflammatory signs or purulent secretion become apparent, AOM or OME become chronic. In contrast to the mild development of OME, chronic otitis media (COM) affects tympanic membrane, ossicular chain and tympanic cavity. Defined as a chronic inflammation of mucosa and submucosa of the middle ear, COM presents with eardrum perforation and erosion of ossicular chain, mastoid, tympanic cavity and the bony cover of the facial nerve. A complication of COM is chronic suppurative otitis media (CSOM) occurring with recurrent ear discharge or otorrhea through the perforated eardrum, persisting longer than three month. Eardrum retraction and cholesteatoma are the consequences. A hearing impairment of up to 40 dB can be observed in children and adults during this course of disease. Causes of AOM are considered as multifactorial and various, including bacterial infections like...
1. GENERAL INTRODUCTION

staphylococcus aureus, gram negative bacteria and pseudomonas aeruginosa or virus infections like influenza or herpes simplex \(^1\), becoming COM in 20% of cases as mentioned above. Therefore, recurrent episodes of middle ear infections and Eustachian tube dysfunction (ETD) at a young age are considered as important factors for the development of COM and CSOM \(^18\). Another key factor in the development and in the course of COM is the auditory tube, thus dysfunctions of this anatomic structure have severe consequences \(^1,4,22\). Adequate equilibration of pressure in the middle ear and the transport of fluids from ME to the nasal cavities being provided by the ET are considered as important. Additionally, the progression of AOM to CSOM is highly affected by the clearing abilities of the ET. If ciliary dysfunction (e.g. primary ciliary dyskinesia) impairs the clearing abilities, bacteria and viruses accumulate in the ME. Furthermore, permanent low pressure in the ME caused by gas resorption of the middle ear mucosa, e.g. an inadequate pressure equalisation via the ET leads to OME \(^1\).

The first step of the development of this disease is the retraction of the eardrum into the tympanic cavity followed by the second and third step: total obliteration of the ME space and retraction of the tympanic membrane onto promontory and the ossicles. The fourth step includes adhesive processes of eardrum and ME. Effusion is the consequence of this vacuum followed by inflammation and oedema if it persists. Mucosal oedema, in turn, closes the orifice of the ET and hampers pressure equalisation. A vicious circle of cause and course sustains the disease. Additionally, each condition obstructing the ET itself or its orifice, like allergic rhinosinusitis, adenoids in front of the pharyngeal or tympanic orifice, tumours of all kinds etc., can lead to ET obstruction and therefore ETD and/or CSOM \(^1,22,26\). As a complication of adhesive otitis media deep retraction pockets in the pars tensa or pars flaccida of the eardrum may occur, containing desquamated keratin debris. This debris does not migrate into the ear canal and can be encapsulated as epidermal inclusions of mastoid or ME. This cyst usually opens in the EAC but cannot be cleaned and stays in position growing in size. The growth of this so-called cholesteatoma is followed by bone erosion of the ossicles and other neighbouring structures obstructing the EAC and inhibiting sound propagation and tympanic membrane mobility \(^26\). A dysfunctional Eustachian tube, however, plays an important role in the cause and persistence of OM but until today, there is still a lack of clearly defined diagnostic criteria \(^27\). The most prominent definition in literature is impaired ability of pressure equalisation in the middle ear solely, although strict clinical definition of ETD would address as well hampered transport of mucous or secretion and insufficient protection against sounds and pathologic germs. An expert panel agreed in 2015 that ETD needs to be classified into dilatory dysfunctions, being divided into functional obstruction, dynamic dysfunction (muscular failure) and anatomical obstruction;
baro-challenge-induced dysfunction and patulous ETD. Additionally the term of ETD should not be used to describe OM, OME, CSOM or tympanic membrane retraction with cholesteatoma. A dysfunctional Eustachian tube may play a profound role in the cause and persistence of these diseases, but is not the diagnose itself. Patients with ETD can have OM but may as well only have symptoms associated with ETD. They complain about “aural fullness”, “popping” in the ear or feeling of discomfort or pain. Clogged ear sensation or the feeling of hearing “under water” e.g. muffled hearing are also common statements as crackling, ringing and autophony, where the patient hears body sounds like breathing and intestinal sounds overloud. However, ETD is often accompanied by OM and should therefore be considered in the diagnostic.

1.4 Diagnostic Tools

For the diagnosis of OM clear criteria are defined and specific symptoms indicate each manifestation. However, for the definite diagnosis these symptoms are not reliable enough and need confirmation by other diagnostic tools since a variety of different illnesses show similar manifest symptoms. Macroscopic and microscopic visualisations of the tympanic membrane are important tools for a first impression. For example the observation of secretion or pus in combination with eardrum perforation gives a strong indication for CSOM. Additionally, tone audiometry showing a conductive hearing loss and impedance audiometric measurements like tympanometry verify the diagnosis.

For ETD the situation appears differently. Just like for the definition, no absolute consent about appropriate and reliable diagnostic tools for ET function or dysfunction is available. Di Martino even stated that none of the available tools are able to draw a full picture of all functions of the ET, always making a combination of different procedures necessary. According to the recommendations of the panel by Schilder et al. the ideal assessment would be a combination of the following diagnostic tools: otoscopy or otomicroscopy of the eardrum, nasopharyngoscopy of the opening of the ET, tympanometry, Rinne’s and Weber’s tuning fork test and/or pure tone audiometry. If the symptoms reported by the patient indicate the occurrence of negative pressure in the middle ear, symptoms like a retracted ear drum via otoscopy and a type C tympanogram via impedance audiometry (e.g. tympanometry) should as well be present for the confirmation of the diagnosis of a dilatory ETD. In contrast to former hypotheses a positive Valsalva or Toynbee manoeuvre can confirm ETD but cannot be considered on an individual basis. In patulous e.g. permanently open ET a tympanic membrane
movement with breathing frequency can be seen in tympanometry and otoscopy \(^{28}\). In addition, the ventilatory functions of ET can be assessed by several other tests, which were summarized by Di Martino \(^{4}\): for the tubomanometry according to Estève, a defined high pressure is applied into the nasopharynx and the change of pressure while swallowing can be measured in the EAC. The sonotubometry can be used no matter if the eardrum is intact or perforated and measures the loudness change of a tone given into the nasopharynx in the EAC. If the ET opens, the tone becomes louder. Another test is the nine-step inflation deflation test, which can only be performed when the eardrum is intact and no effusion is present \(^{4}\). High and low pressure is applied into the EAC and the patient is asked to equalise the therefore altered pressure in the middle ear via swallowing. During the swallowing process several measurements are performed. The test shows the ability of the ET to equalise pressure in different situations. During the forced response test through a perforated eardrum the pressure in the middle ear is increased and the opening pressure of the auditory tube, e.g. the restoring forces of the adhesive mucosa of the ET are defined \(^{4}\). Active and passive tubal functions can be assessed but the practical use is questionable. For experimental questions measurements in the pressure chamber can be performed, due to the possibility to generate different pressure situations of the surrounding. Especially baro-challenge-induced ETD can be provoked \(^{4}\). Conventional radiographic imaging, computed tomography or magnet resonance tomography are as well suitable to exclude abnormal findings like anatomical malformations or obstructions of the tubal orifice by tumours or adenoids. However, it does not belong to the routinely performed diagnostic examinations and can be used in cases where these pathologies are suspected \(^{28}\). Because of its relevance for this thesis the tympanometry will be described briefly in the following passage.

Impedance audiometry e.g. tympanometry is a rapidly performable objective measurement of several parameters of the middle ear. The obtained results are reliable, reproducible and the conduction of the measurement can easily be delegated to the medical assistant \(^{4}\). They give evidence about eardrum integrity, eardrum compliance, disruption of the ossicular chain, occurrence of middle ear effusion and middle ear ventilation e.g. indirectly therefore about ET functions. A three-canaled probe is placed into the EAC, containing a microphone, a loudspeaker and a pressure pump with manometer (Figure 1.4) \(^{29}\).
1. GENERAL INTRODUCTION

Figure 1.4 Principle of tympanometry. The three-canaled probe contains a microphone (M), a pressure pump with manometer (PP) and a loudspeaker (L), giving the probe tone into the external auditory canal (EAC) and measuring its reflection from the eardrum (ED).

The probe hermetically seals the EAC and emits a tone in the frequency of 226 Hz (1000 Hz for infants) to induce oscillation of the eardrum. The reflected tone is recorded by the tympanometer. This procedure is performed at different air pressures typically between 200 and -400 daPa. This change of pressure is usually conducted with a speed of 200 daPa/s. During the measurement the pressure at which the eardrum vibrates best is determined. This is the case if the pressure in the middle ear resembles the outer ear pressure and thus, the tympanic membrane reflects least of the given probe tone. The achieved tympanograms can be classified according to a scheme provided by Jerger in 1970, which is still in practical use until today.

The parameters used to classify the patterns are the volume of the external ear canal (ECV), the static admittance or peak of compliance (SA), the pressure at maximum compliance (TTP) and the tympanometric width (TW) (Figure 1.5). Following this scheme, a type A tympanogram represents the physiological status of the middle ear, indicating an accurate pressure equalisation by the ET. The ECV is between zero and 2.5 mL with a single SA between 0.37 mmho and 1.66 mmho (adults >80 up to 2.5 mmho). The TTP varies between -100 daPa and 100 daPa. Between 48 daPa and 134 daPa (up to 234 daPa in infants) should be the TW. In a type B tympanogram either no clear peak or a flat tracing corresponding with a variable ECV occurs. Various causes of this flat tracing are reported for which the ECV can be used as interpretation tool.
Figure 1.5 Schematic depiction of measuring parameters in a type A tympanogram of the sheep. Static admittance (SA), tympanometric width (TW), pressure at maximum compliance (TPP) and ear canal volume (ECV) were analysed.

Is the ECV approximately zero, the probe is either forced against the ear canal wall or clogged by ear wax. Associated with a non-zero ECV (>0.3 mL) up to 2.5 mL is tympanic effusion occurring for example in middle ear inflammation or ETD. An increased ECV of more than 2.5 mL is considered as evidence for tympanic membrane perforation. Type C tympanograms differ from type A tympanograms by a TPP of more than 100 daPa or less than -100 daPa, indicating a non-physiological pressure in the middle ear. Indirectly this indicates a dysfunctional auditory tube.

The SA is increased in a type D tympanogram exceeding 1.66 mmho, which can be interpreted as a hypermobile, atrophic or scarred, but otherwise normal tympanic membrane. According to Di Martino and older sources in literature in this curve type, w-shaped notches can be present. In practical use this phenomenon is rarely observed, due to the fact that the range of the preset scale allows no complete depiction of the tympanogram and it appears to be “open” showing no peak. Is the tympanograms’ width not in the physiologic range but considerably wider, the tympanogram is considered as type E, being associated with ossicular chain disruption.
1.5 Therapy

The current management of OM depends on the manifestation of the disease. The treatment of AOM is symptom related, controlling fever and otalgia in combination with decongestant nasal spray. Antibiotics are only recommended when the infection was confirmed including a resistance screening. In OME a spontaneous healing rate of up to 42%\(^1\) can be observed after 6 month. Thus, if no complications are developed, conservative treatment of symptoms can be applied. Still, if the described condition persists longer than 3 month, surgical intervention via myringotomy and ventilation tubes inserted into the eardrum is recommended. If the patient presents with recurrent episodes, adenoidectomy is advisable. In contrast to OME, in CSOM the initial therapy aims at optimization of the present condition for the surgical intervention regarding tympanoplasty and ossiculoplasty. The reduction of pathogens and the complete drying of the external and middle ear are the goals of the therapy. Currently the therapy includes the application of antiseptic and antibiotic eardrops, in addition to regular cleaning of the EAC. The surgical treatment aims at permanent eardrum closure, healing of inflammation and drying of the ear to increase hearing abilities. In general the applied treatment depends on the age of the patient and the occurring complications\(^1,18,19\).

In addition to the symptomatic treatment of otitis media, restoration of ET functions plays an important role in the management of this disease if one of the causes can be found in a dysfunctional ET. Therefore, the treatment especially of occurring dilatory ETD is as well essential for the long-term healing process\(^19,27\). Generally mild dilatory ETD resolves after a few days with the induction of increased opening frequency of the ET via yawning, Valsalva manoeuvre, swallowing or chewing. If the dysfunction persists, non-surgical treatment options are available. One option is nasal douching with saline solution to remove debris and excess of mucus from the nasal sinuses\(^27\). Decongestants, antihistamines, or nasal or oral corticosteroids are used to reduce swellings and inflammation. Additionally, antibiotics against occurring rhinosinusitis are given. If these non-surgical interventions fail, subsequent surgical therapy follows. The current goldstandard of treatment is the insertion of ventilation tubes or grummets into the eardrum, just as in OME. However, extrudation after six to nine month and crusting of the tube can be observed, with a scarred eardrum if frequently reused as long-term consequence\(^27\). Another upcoming method is the balloon dilatation of the ET, where under nasal endoscopic vision a balloon catheter is expanded in the ET to loosen obstructions and adhesions in the mucosa\(^31\). Transtubal application of fluids as well as Eustachian tuboplasty, where massive occurrence of mucosa and cartilage is removed, is used to improve the functions of the ET in
regard to ventilation. The choice of treatment is based on the specific symptoms and situation of the patient as well as the invasiveness of the method and the surgical preference of the surgeon. The effectiveness of each method is hardly definable, due to the fact that lacking consent on the definition of ETD and inadequate study conditions impede the analysis.

1.6 Animal Model

The mammalian ear in general shows the same components for the process of hearing: the EAC, the tympanic membrane, the three ossicular bones with attached tendons and muscles, the tympanic cavity and a similar course of nerves. However, large variations in size and in some cases in the formation of each specific structure between the mammalians (including man) can be found. Regarding the middle ear, the anatomy of the sheep is morphologically equivalent to human ears. The tympanic membrane in the sheep shows the same parts as the tympanic membrane in humans but the pars flaccida is remarkably larger. In contrast to these findings, the surface of the eardrum measures only half the size in humans and is more ovally shaped (Figure 1.6).

Figure 1.6 Outlines of the tympanic membrane of men and sheep with pars flaccida (grey) and pars tensa (white) with the manubrium mallei (black line) modified according to Fay et al.

The tympanic cavity, especially the hypotympanon measures almost twice the size and is called “bulla”. The human middle ear lacks such a structure. The other elements of the sheep’s middle ear are at least two third in size of the human structures. Therefore, the sheep is considered as suitable for the training of manual skills of surgeons and the purpose of middle ear implant research. However, the ET of the sheep has already been evaluated in regard to histology in 1971.
Among the species of man, opossum, mole, bat, cat, rat, rabbit and horse, the ET of the sheep was analysed in regard to epithelium, goblet cells, connective tissue framework and the occurrence of the diverticulum neighbouring the pharyngeal orifice. Occurring epithelium, goblet cells and the existence of a diverticulum, although smaller than in humans, were similar. The goblet cells were differently spread and unlike in horses and humans the connective tissue framework contained no cartilaginous fragments. The dimensions of the ET were assessed in a recent study, showing that the distance from the pharyngeal orifice to the isthmus and the isthmus height, both important for implanting the auditory tube, are very similar in men and sheep. The total length of the ET as well as the distance from middle ear orifice to isthmus are larger in humans.

In addition to the anatomical consideration, specific breeds of sheep are well domesticated and can easily be handled and trained for specific tasks. Even though pigs can also be trained for specific purposes, the hectiness and loudness in vocalisation of this species aggravates periods of keeping still and remaining quiet. The sheep, in contrast, may be easier to train to perform the tasks needed for example in impedance audiometric measurements.

In conclusion, the anatomical characteristics and their similarities to human anatomy predestine the species of the large animal sheep for research in middle ear and auditory tube implants. The implant design, which shall be tested in the sheep and will be produced for human application, may be kept and no adjustment for clinical studies might be needed. The methods used for answering research questions may therefore also be transferred bidirectional between the species with small restriction and modifications according to the species characteristics. Furthermore, the attributes of training and easy handling allow the use of methods where the sheep needs to work with the researcher to achieve results.

Therefore, the aim of the first study of this thesis (Chapter 2) was to establish tympanometry in conscious sheep to provide a test of objective evaluation of eardrum integrity, middle ear ventilation and functioning of the auditory tube. The objective for the second study (Chapter 3) was to assess the feasibility of in vivo implantation of a commercial coronary stent from the nasopharynx into the ET and to evaluate the efficiency of the stent for the maintenance of patency and function of the ET. Evaluation of induced aseptic otitis media was as well performed as possible disease model. In addition, implanting procedure as well as the examination of the specific structures via different pre- and post-mortem tests were needed and needed to be adapted to this species to establish this animal model for the evaluation of stents.
and other implants in the ME and ET. This battery of tests will be discussed in detail in this thesis.
2. Tympanometric measurements in conscious sheep - a diagnostic tool for pre-clinical middle ear implant studies.

Friederike Pohl¹, Gerrit Paasche¹ ², Thomas Lenarz¹ ², Robert Schuon¹

¹ Department of Otolaryngology, Hannover Medical School, Hannover, Germany
² Hearing4all cluster of excellence, Hannover Medical School, Hannover, Germany

Abstract

OBJECTIVE:
To investigate and establish the use of tympanometry in conscious sheep to provide a means of objective assessment of tympanic membrane integrity, middle ear ventilation and functioning of the Eustachian tube (ET).

DESIGN:
After conditioning the sheep for four weeks, tympanometric measurements at 226 Hz were carried out weekly for 13 weeks. Before measurements, the external ear canal had been cleaned. Resultant curves were classified according to human reference values.

STUDY SAMPLE:
Tests were performed on 12 female blackface sheep.

RESULTS:
After cleaning of the external ear canal under otoscopic control, tympanic membranes were intact with no evidence of acute or chronic middle ear inflammation, middle ear effusion or retraction. Cleaning ensured valid, objective and reproducible measurements. As the majority of normal tympanograms were notched without the appearance of any malformation, an additional tympanogram type (AN) was introduced. The notched appearance can most likely be explained by the anatomy of the middle ear of the sheep and the test frequency that was used.

CONCLUSION:
The current study demonstrated how tympanometry can be used to evaluate treatment modalities for middle ear and ET function in conscious sheep. This provided a large animal model for further human research in otology.

KEYWORDS:
Animal studies; anatomy; medical audiology; middle ear; physiology

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The student performed the planning of the scientific design, the animal experiments, analysis and evaluation of the data including scientific writing.
3. Stenting the Eustachian Tube to Treat Chronic Otitis Media – a Feasibility Study in Sheep

Friederike Pohl¹,², Robert Alexander Schuon¹,², Felicitas Miller¹,³, Andreas Kampmann³, Eva Bültmann⁴, Christian Hartmann⁵, Thomas Lenarz¹,², Gerrit Paasche*¹,²

¹ Department of Otolaryngology, Hannover Medical School, Hannover, Germany
² Hearing4all cluster of excellence, Hannover Medical School, Hannover, Germany
³ Clinic for Cranio-Maxillo-Facial Surgery, Hannover Medical School, Hannover, Germany
⁴ Institute of Diagnostic and Interventional Neuroradiology, Hannover Medical School, Hannover, Germany
⁵ Department of Neuropathology, Hannover Medical School, Hannover, Germany

Short title: Stenting the Eustachian Tube in Sheep

* Corresponding author

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The student contributed to the scientific design of the animal experiment and performed animal experiment, histological processing, analysis and evaluation of the data including scientific writing.
Abstract

Untreated chronic otitis media severely impairs quality of life in affected people. Local destruction of the middle ear and therefore hardness of hearing and hearing loss can be the consequences. Thus, a large animal model for testing possible implants designed for human application might be beneficial. The objectives in this study were to evaluate the feasibility of the insertion of a commercial coronary stent from the nasopharynx into the Eustachian tube in vivo and to analyse the efficiency of the stent to maintain the patency and function of the Eustachian tube. Furthermore, induced aseptic otitis media shall be evaluated as possible disease model.

Bilateral implantation of bare metal cobalt-chrome coronary stents of two sizes was endoscopically performed in three healthy blackface sheep using a nasopharyngeal approach. A perforated catheter was used to apply prostaglandin $E_2$ and platelet activating factor as inflammatory mediators.

Stent implantation into the Eustachian tube was feasible without any intra- or post-operative complications. The health status remained unaffected and no signs of inflammation in connection to the applied mediators were observed. All stents preserved their cylindrical shape. All shorter stents remained in position and ventilated even though partly filled with secretion or tissue. One of the long stents was dislocated towards the nasopharynx. Both others remained fixed at the isthmus but appeared blocked by tissue or secretion. Tissue had grown on top of the struts of all stents with reparation of the tissue lumen interface.

The procedure of stenting the Eustachian tube was transferred from cadaver studies to in vivo application without any complications. The stent was well tolerated and promoted ventilation of the middle ear and clearance of the auditory tube. It seems to be sufficient to place it only in the cartilaginous part of the Eustachian Tube.

Introduction

Acute and chronic otitis media, affecting the middle ear and therefore the ability of hearing, still is an issue in human medicine [1]. Especially otitis media with effusion (OME) is not only common in children under the age of ten, but the most prevalent reason why advice and treatment by an otorhinolaryngology specialist is needed [2]. In approximately 20% of patients symptoms become chronic [2], causing severe and often irreversible damage of middle ear structures like eardrum and ossicular chain. In the development of these diseases the Eustachian or auditory tube (ET) and its anatomy are one of the key factors [2-3].
The ET forms the only connection between middle ear and nasal cavities. It consists of an inelastic bony part starting at the protympanon of the middle ear and covering one third of the ETs full range in humans. This merges into an elastic cartilaginous part, which extends over the other two thirds and ends in the nasopharynx. The conjunction of both parts creates a narrow passage, the isthmus [4]. The most important functions of the ET are transport of secretion, protection against pathologic germs and middle ear ventilation [3]. If one or more of these functions cannot be maintained, a dysfunctional auditory tube (ETD) and middle ear effusion followed by middle ear inflammation can be consequences. The causes of this functional impairment are diverse, just as their treatment.

To treat ETD and otitis media with effusion different approaches such as a PVC tube with an attached thread inserted via the perforated tympanic membrane [5], a Silastic® tube, inserted via the tympanic orifice of the ET [6] or a gold wire staying in the ET for years with rare rejection and initially good functions [7] were applied in patients but with limited long-term success [8-9].

The current management of this symptom complex includes conservative methods like the Valsalva manoeuvre for pressure equalisation; nasal douching with saline solution or nasal application of decongestants, antihistamines, or corticoids. The most common surgical approach is the insertion of a tympanostomy tube into the eardrum [10].

Apart from this two more approaches are used: Eustachian laser tuboplasty, where enlarged mucous membranes and cartilage are removed to avoid obstruction [11], and balloon dilatation, where a balloon catheter is inserted into the cartilaginous part of the ET and inflated with high liquid pressure to loosen adhesions and dilate the lumen [12]. Additionally, the topical application of fluids directly into the auditory tube [13] recently emerged in research and for practical use.

Despite the fact that these numerous methods appear in practical use and literature, according to Llewellyn et al. [10] there is little consensus about indications of treatment and moreover, conclusions regarding efficacy are questionable. Also the causes of dysfunction and the mechanisms of intervention and long-term clinical outcomes need to be assessed more profoundly [14].

In preclinical research in chinchilla and rabbit a poly-L-lactide Eustachian tube stent without adaptation to the size of the animal was implanted through the bulla and tympanic membrane and investigated but with moderate outcome [15-16]. Additionally, the sheep was evaluated for preclinical assessment of middle and inner ear implants [17], and the possibility of endoscopic implantation of a commercial coronary stent through the nasopharyngeal orifice of the ET was proven in a cadaver study [18], for being able to test human sized stents in a large animal model.
To investigate the feasibility of this approach also in vivo, in the present study differently sized commercial coronary stents were implanted into the auditory tubes of blackface sheep.

In addition, it would be beneficial to have an adequate disease model available. There are models described in the literature either using cauterization of the ET [19], knock-out mice [19] or Streptococcus pneumonia in the rat [20]. In chinchilla aseptically triggered OME was induced [21] with inflammatory mediators: platelet activating factor (PAF) [22] and Prostaglandin E$_2$ [21]. This provides an easy to apply, reversible method without the risk of uncontrolled infection of the animal.

Therefore, the objectives of the current study were to evaluate the feasibility of the in vivo insertion of a commercial coronary stent from the nasopharynx into the Eustachian tube and to analyse the efficiency of the stent to maintain the patency of the Eustachian tube e.g. to promote ventilation of the middle ear, protection against pathologic germs and transport of secretion. Furthermore, induced aseptic otitis media shall be evaluated as possible disease model.

**Material & Methods**

**Ethic statement**

The State Office for Consumer Protection and Food Safety, Dept. of Animal Welfare in accordance with the German and European animal welfare legislation approved this study under the number 12/1089. With regard to the valid directives for accommodation, care and usage of experimental animals, the animals were taken care of and the experiments were performed in a central animal facility.

**Stents**

ProKinetik Energy® stents (Biotronik, Berlin, Germany) consisting of a non-degradable cobalt-chrome alloy with a strut thickness of 60 µm and a recoil of less than five percent were implanted in two different sizes: 2.75 mm x 26 mm (left ear) and 2.0 mm x 20 mm (right ear). The stents were mounted on an expandable balloon catheter (Rapid exchange catheter, length: 1.4 m).

**Animals and study design**

Auditory tubes of three healthy adult (2 to 4 years) female blackface sheep were stented bilaterally with one week delay between both sides. One week prior to the first implantation a first bilateral control of external and middle ears and endoscopic examination of the nasopharyngeal orifices of the ET was performed under general anaesthesia. At the time of first implantation, a sterile
middle ear inflammation was triggered in the second ear followed by implantation of the second side one week later. Regular endoscopic controls of the nasopharyngeal orifices of the ET were performed according to the study design (Fig 1). A second sterile inflammation was induced in the first implanted ear one week before sacrifice after 12 weeks. The degree of inflammatory reaction was evaluated by a daily check on the general health of the animals and an endoscopic score of the pharyngeal orifice of the ET as described below.

Figure 1 Study design in weeks. Arrows indicate time points of general anaesthesia with endoscopic control (white), instillation of inflammatory mediators (red) or stent implantation (green).

General anaesthesia, implantation and induction of inflammation

Implantation as well as induction of inflammation was performed under general anaesthesia (GA) after sedation with midazolam (0.2 mg/kg i.v.; Midazolam-ratiopharm® 5 mg/mL, Ratiopharm, Blaubeuren, Germany) and induction of GA with propofol (5-10 mg/kg i.v.; Propofol®-Lipuro 10 mg/mL, B. Braun Melsungen AG, Melsungen, Germany). For the maintenance of GA, isoflurane (1.5-2.0% end-tidal inhalation; Isofluran CP 1 mL/mL, CP-Pharma, Burgdorf, Germany) was used. To prevent bleeding and to provide local anaesthesia, pointed swabs wetted with Naphazolin 10 mL (Privin® 1mg, Novartis, München, Germany) and Lidocaine 5 mL (Xylocain 2%, AstraZeneca GmbH, Wedel, Germany) were applied into both nostrils prior to the endoscopic approach.

Stenting was performed via a nasopharyngeal endoscopic approach of the Eustachian tube (ET). The stent, mounted on the balloon catheter, was completely inserted into the epipharyngeal
orifice of the ET via the working canal of a flexible broncho-fiberscope (Broncho-Fiberskop: 3.7 mm diameter, 1.5 mm working canal, 0° angle of view, 110° opening angle, 54 cm length, Karl Storz, Tuttlingen, Germany). With physiologic saline solution the balloon was inflated and thus the stent expanded, using a pressure of 10 bar for two minutes. Afterwards the balloon was deflated, held in position for one minute and slowly extracted from the orifice of the ET. Whilst the short stent (2.0 mm x 20 mm) stayed only in the cartilaginous part of the ET, the longer stent (2.75 mm x 26 mm) was fixed by clamping it into the isthmus between bony and cartilaginous part.

Inflammation was initiated using platelet activating factor $10^{-5}$ mol/L (1-O-Hexadecyl-2-O-acetyl-sn-glycero-3-phosphocholine, Bachem GmbH, Weil am Rhein, Germany) and prostaglandin E$_2$ $10^{-5}$ mol/L (Prostaglandin E$_2$, Sigma-Aldrich Chemie, Schnelldorf, Germany) delivered as a single treatment of 1 mL via a perforated balloon catheter at the time of first implantation in the unstented right ear and in double concentration one week before sacrification in the stented left ear. Postoperative pain management was provided by Carprofen 1.4 mg/kg i.v. (Rimadyl®, 50 mg/mL, Pfizer, Berlin, Germany) and protection from bacterial inflammation performed with Benzylpenicillin-Dihydrostreptomycin 0.04 mg/kg s.c. (Veracin Comp.®, Albrecht GmbH, Aulendorf, Germany).

**Health and endoscopic score**

To ensure the animal’s health, a daily check on their general health status and periodical endoscopic examinations of the ear and ET according to Fig 1 were done. The health score according to [23] was modified and included parameters like breathing frequency, rumination, intake of food and water, head tilt or nasal discharge and reached from zero (unaffected constitution) to seven (severely affected constitution) (Table S1). The quality of mucus, the degree of opening of the nasopharyngeal orifice of the ET, inflammatory erythema and swelling were assessed in the endoscopic score (Table 1). A maximum score of 12 (severe inflammation) and a minimum of zero (no inflammation) were possible, specifying a score of zero to three as non-inflammatory, from four to six as mild inflammation, from seven to nine as moderate inflammation and from ten to twelve as severe inflammation. Additionally, the visibility of the implanted stent in the proximity of the ET opening was documented. Examples for specific endoscopic images are presented in Fig 2.
3. STENTING THE EUSTACHIAN TUBE TO TREAT CHRONIC OTITIS MEDIA

Figure 2 Endoscopic view of ET for the evaluation of stent position and inflammation via the endoscopic score.

Examples of (A) no [score 1-3], (B) mild [score 4-6] and (C) moderate [score 7-9] inflammation in the region of the nasopharyngeal orifice are provided.

Table 1 Score for semi quantitative evaluation of the endoscopic images of the nasopharyngeal orifice of the ET.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Endoscopic score value</th>
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<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Quality of mucus</td>
<td>0</td>
</tr>
<tr>
<td>Opening degree of n. orifice*</td>
<td>0</td>
</tr>
<tr>
<td>Inflammatory erythema</td>
<td>0</td>
</tr>
<tr>
<td>Inflammatory swelling</td>
<td>0</td>
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*n. orifice = nasopharyngeal orifice

Fixation, embedding and staining procedure

After 12 weeks of implantation, animals were sacrificed under GA by an overdose of pentobarbital i.v. (Release® 300 mg/mL, WDT, Garbsen, Germany) before decapitation behind the second cervical vertebrae.

Post-mortem, a spiral computed tomography (CT) scan was performed. On coronal reconstructions the stent in its entire length as well as the middle ear and the external auditory canal were depicted (example shown in Fig 3). The location of the stent and the degree of its obstruction (tissue or secretion) were examined. The middle ear, including hypo-, meso- and epitympanon was evaluated regarding tissue formation and occurrence of effusion.
For histologic analysis, each ET with surrounding tissue was dissected orienting on the nasopharyngeal opening and the opening to the middle ear, using a bone saw (FK23 bone saw, Bizerba, Balingen, Germany). The specimen were washed in physiological saline solution (B. Braun Melsungen AG) and fixed in formalin (3.5%, pH 7.4; C. Roth, Karlsruhe, Germany) for two weeks. Prior to embedding in methylmethacrylat (MMA; Merck KGaA, Darmstadt, Germany) [24], dehydration of the specimen via an increasing ethanol series (70%, 80%, 90%, 100%; Merck) was done. Each step was performed over night and finalized with MMA infiltration and polymerisation in a water bath with increasing temperature (35°C to 40°C) for two to four days, depending on the status of polymerisation. The excess MMA was removed with a plaster model trimmer (HSS 88, Wassermann Dental-Maschinen GmbH, Hamburg, Germany) until only the specimen were left. Specimen were cut into two halves and fixed on a specimen holder to cut slices of approx. 33 µm thickness with a saw microtome (Leica SP1600®, Leica Biosystems, Wetzlar, Germany), beginning in the middle of each ET following the course of the Eustachian tube in both directions. Additionally, slices of approx. 1 mm thickness were discarded in periodical intervals. Staining of the slices was performed with alizarin red (Alizarin red S staining solution; Merck,) and methylene blue (Löffler’s methylene blue solution; Merck). The slices were incubated for 45 s with methylene blue on a heating plate (80°C). After rinsing with distilled water, the slices were incubated with alizarin red for 1.5 minutes. Drying in an incubator at 37°C over night followed an additional rinsing step. After staining each slice was mounted on microscopic slides by using Entellan®-new (Merck) and covered by cover slips.
3. STENTING THE EUSTACHIAN TUBE TO TREAT CHRONIC OTITIS MEDIA

Figure 3 Coronal CT sections of both stents (*) in situ for each animal.

Tympanic cavity (ME), nasopharynx (NP) and spinal canal were used for orientation and evaluation. The red lines indicate the regions of histologic evaluation.

Histologic analysis and evaluation

Histologic analysis was performed with an image editing software (NIS-Elements Imaging Software 4.20®, Nikon, Düsseldorf, Germany) after digitalisation of the histologic slices under a microscope (SMZ1000®, Nikon, Düsseldorf, Germany with a Nikon Digital Sight DS-Vi1 camera) at 2x magnification. In each set of slices the end of the stent in proximity to the nasopharynx was set as starting point for the analysis and the end of the stent in proximity to the middle ear opening as the end point of analysis. The position of the discarded slices was taken as reference to estimate the length of the tube and to divide the length of the stent in the tube into four parts. Part one represented the first third of the cartilaginous part of the ET and the
3. STENTING THE EUSTACHIAN TUBE TO TREAT CHRONIC OTITIS MEDIA

beginning of the stent, following it from the nasopharyngeal opening. Part two adjoined it representing the middle part, whereas part three covered the final third of the cartilaginous part of the tube in direction of the middle ear. Due to the different lengths of the stents, the fourth part overlapped in parts with the third part in the shorter stents but was positioned in the isthmus region for the longer stents and revealed the ending of each stent. In each part three representative concurrent slices were analysed. In each slice, the lumen (L) of the auditory tube, the amounts of secretion (S) and tissue (T) and the total region of the Eustachian tube excluding chondral and bony parts, gland tissue or fat (ROI) were assessed. The free lumen \( L_F \) was calculated by subtraction of the secretion from the lumen. Tissue, lumen, secretion and free lumen were given as percentage from the total ROI. The values of the three consecutive slices were averaged to receive the results for a specific section of the ET. To calculate overall values averaged results were used.

An ellipse was positioned on the slices, such that most visible struts of the stent were on or very close to the ellipse (Fig 4). The stent diameter was determined via the stent area (the area of the ellipse) in the histological sections. The area was quantified in the slices for the four different parts of the ET and was compared to the references given by the manufacturer for each stent.

Figure 4 Slice of the ET with plotted ellipse for the analysis of stent expansion.
Depicted are Rüdinger’s safety canal (RC), auxiliary gap (AG), the tubal cartilage (C) and free lumen \( L_F \) of ET. Bone is coloured red; mucosa, tissue, fat and glandular tissue blue and cartilage violet. An ellipse is depicted on the struts (*) indicating the position of the stent.
Results

Stent implantation and application of inflammatory mediators

In all three animals, the endoscopic approach revealed a free view on the pharyngeal orifice (Fig 2), and implantation was easily feasible on both sides. Furthermore, the insertion of the entire stent could be done without complication for both stent sizes. The application of inflammatory mediators using a perforated balloon was feasible in the right auditory tube prior to implantation and not hampered by the already implanted stent in the left ET at the end of the study.

Health score and endoscopic score

During the entire period of the experiment all three animals displayed a health score of two or less from a maximum score of seven (Table 2). Recurring periods of hot weather resulted in a 0.5 points increase due to enhanced breathing frequency of the sheep. In animal three, serous nasal discharge was observed three times: two weeks prior to the first manipulation, during the week after the first general anaesthesia before stent implantation and triggering of inflammation, and in week three after stent implantation and first application of inflammatory mediators.

The mean endoscopic score revealed no inflammatory signs for two of the short and one of the longer stents (Table 2, Fig 5). Mild inflammatory reaction occurred with one of the short and one of the long stents. A moderate reaction was detected for one long stent (Table 2, Fig 2(C)). Sporadic visibility in the proximal orifice was apparent in all implanted stents. In animal one, the long stent was visible in each performed control and in animal two in four of the seven endoscopic controls (Table 2). In all three animals an increase in secretion in the region of both ETs was detected in comparison to the first GA before implantation. Neither in the health score nor in the endoscopic score (Fig 5) severe inflammation was detected in all three animals showing a temporal connection to the instilled inflammatory mediators.
Figure 5 Achieved endoscopic score values for (A) left (long stent) and (B) right (short stent) ET of each sheep under general anaesthesia in the course of the experiment. In the first GA (week -1) a score of zero was observed.
### 3. STENTING THE EUSTACHIAN TUBE TO TREAT CHRONIC OTITIS MEDIA

<table>
<thead>
<tr>
<th>Health score (0-7)</th>
<th>Sheep 1</th>
<th>Sheep 2</th>
<th>Sheep 3</th>
</tr>
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<tr>
<td>Mean: 0.16</td>
<td>Mean: 0.14</td>
<td>Mean: 0.19</td>
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<td>Max: 0.5</td>
<td>Max: 2</td>
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<tr>
<th>Stent size</th>
<th>2.75 mm x 26 mm (left)</th>
<th>2.0 mm x 20 mm (right)</th>
<th>2.75 mm x 26 mm (left)</th>
<th>2.0 mm x 20 mm (right)</th>
<th>2.75 mm x 26 mm (left)</th>
<th>2.0 mm x 20 mm (right)</th>
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|-------------------|-------------------------|------------------------------------------------------|---------------------|--------------------------------------------------|---------------------|--------------------------------------------------|---------------------|--------------------------------------------------|

<table>
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<tr>
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<th>With stent: Possible, fluid leakage from prox. orifice</th>
<th>Without stent: Possible, fluid leakage from prox. orifice</th>
<th>With stent: Possible, fluid leakage from prox. orifice</th>
<th>Without stent: Possible, fluid leakage from prox. orifice</th>
<th>With stent: Possible, fluid leakage from prox. orifice</th>
<th>Without stent: Possible, fluid leakage from prox. orifice</th>
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</thead>
</table>

<table>
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<td>Mean: 1.13</td>
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<td>Max: 8.0</td>
<td>Max: 5.0</td>
<td>Max: 2.0</td>
<td></td>
</tr>
<tr>
<td>Min: 0.0</td>
<td>Min: 0.0</td>
<td>Min: 0.0</td>
<td></td>
</tr>
<tr>
<td>Stent visible: 7/7</td>
<td>Stent visible: 0/7</td>
<td>Stent visible: 4/7</td>
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</tr>
<tr>
<td></td>
<td>Stent visible: 3/7</td>
<td>Stent visible: 2/7</td>
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<table>
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<tr>
<th>CT Scan</th>
<th>Stent position: Prox. distorted</th>
<th>Stent position: Cartilaginous part</th>
<th>Stent position: Cartilaginous part</th>
<th>Stent position: Cartilaginous part</th>
<th>Stent position: Cartilaginous part</th>
</tr>
</thead>
<tbody>
<tr>
<td>M &amp; E-Tym:</td>
<td>Minimal soft tissue</td>
<td>Stent lumen: Obstructed</td>
<td>Stent lumen: Obstructed</td>
<td>Stent lumen: Obstructed</td>
<td>Stent lumen: Obstructed</td>
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<tr>
<td>Minimal soft tissue</td>
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<tr>
<th>Position during experiment</th>
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<tr>
<td>Prox. dislocation of about 25% of length</td>
<td>As inserted</td>
<td>As inserted</td>
<td>Slight proximal dislocation</td>
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<th>Histological analysis (all four parts)</th>
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<th>Sheep 2</th>
<th>Sheep 3</th>
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<tbody>
<tr>
<td>ROI: 100% / 12.35 mm²</td>
<td>ROI: 100% / 9.82 mm²</td>
<td>ROI: 100% / 9.76 mm²</td>
<td>ROI: 100% / 9.50 mm²</td>
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<tr>
<td>T: 73.44% / 9.07 mm²</td>
<td>T: 69.35% / 6.81 mm²</td>
<td>T: 75.10% / 6.02 mm²</td>
<td>T: 61.65% / 3.33 mm²</td>
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<tr>
<td>L: 26.56% / 3.28 mm²</td>
<td>L: 30.65% / 3.01 mm²</td>
<td>L: 24.90% / 1.50 mm²</td>
<td>L: 38.33% / 3.74 mm²</td>
</tr>
<tr>
<td>S: 11.84% / 1.46 mm²</td>
<td>S: 13.26% / 1.3 mm²</td>
<td>S: 13.55% / 0.82 mm²</td>
<td>S: 0.94% / 0.09 mm²</td>
</tr>
<tr>
<td>Lé: 14.72% / 1.82 mm²</td>
<td>Lé: 17.39% / 1.71 mm²</td>
<td>Lé: 11.35% / 0.69 mm²</td>
<td>Lé: 37.41% / 2.11 mm²</td>
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<table>
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<th>Stent dimensions (mm²)</th>
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<th>Sheep 3</th>
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<tr>
<td>Mean: 5.22</td>
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<td>Mean: 5.36</td>
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<tr>
<td>Max: 6.37</td>
<td>Max: 3.56</td>
<td>Max: 3.65</td>
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</tr>
<tr>
<td>Min: 2.85</td>
<td>Min: 1.92</td>
<td>Min: 3.03</td>
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<th>Ref. left: 5.94 mm²</th>
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<td>Min: 3.03</td>
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<table>
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<tr>
<th>Stent dimensions (mm²)</th>
<th>Sheep 2</th>
<th>Sheep 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 2.26</td>
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<tr>
<td>Max: 3.70</td>
<td>Min: 3.03</td>
<td></td>
</tr>
</tbody>
</table>

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### 3. STENTING THE EUSTACHIAN TUBE TO TREAT CHRONIC OTITIS MEDIA

Table 2 Summary of the specific findings for each sheep.

<table>
<thead>
<tr>
<th>Interface mucosa &amp; lumen</th>
<th>Ciliated epith.: yes</th>
<th>Prismatic epith.: yes</th>
<th>Ciliated epith.: yes</th>
<th>Prismatic epith.: yes</th>
<th>Ciliated epith.: yes</th>
<th>Prismatic epith.: yes</th>
<th>Ciliated epith.: yes</th>
<th>Prismatic epith.: yes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Struts in lumen (%)</td>
<td>17.5</td>
<td>43</td>
<td>19.5</td>
<td>26</td>
<td>6.5</td>
<td>40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Distal = in direction of middle ear; Prox. = Proximal = in direction of nasal cavities; M & E-Tym = meso- and epitympanon; H-Tym. = hypotympanon; Epith. = Epithelium; ROI = entire Eustachian tube, excluding bony and chondral parts; L = lumen; S = secretion; T = tissue; Lf = free lumen
CT scans
Coronal reconstructions (Fig 3) demonstrated ventilated middle ears with few or minimal accumulation of secretion and few soft tissue formation. The CT scans displayed air filling with proximal obstruction in all short and one long stents. In contrast to this the remaining longer stents appeared fully obstructed. All stents were located in the chondral part of the ET. In animal one, the long stent showed a proximal dislocation in direction of the nasal cavities, and in animal two the shorter stent was located directly in the nasopharyngeal orifice of the ET. Both accurately positioned longer stents showed narrowing in the bony part e.g. isthmus region of the ET.

Histologic analysis
Tissue formation was assessed in the histologic analysis. The ET stented with the 2.75 mm x 26 mm sized implant covered on average an area (ROI) of 10.2 ± 2 mm² with 6.6 ± 2.2 mm² tissue (T) and 3.6 ± 0.3 mm² lumen (L). The lumen was filled with 1.2 ± 1 mm² secretion (S), leaving a free lumen (L̄) of 2.4 ± 1.1 mm². The ET implanted with the smaller stent showed a ROI of 7.2 ± 2.2 mm² with 4.9 ± 1.8 mm² tissue and 2.4 ± 0.8 mm² lumen, the latter filled with 0.8 ± 0.4 mm² secretion, leaving a free lumen of 1.5 ± 0.7 mm². The values for each animal are summarized in Table 2. When evaluating the tissue in the different parts (1 to 4) of the tube, in both ETs an increase in tissue as well as a decrease in lumen, secretion and free lumen was detected from nasopharynx (part 1) to middle ear (part 4) (Fig 6) with only minor differences between parts 1 to 3 (not shown).

Figure 6 Areas of tissue occurrence (T), lumen (L), secretion (S) and free lumen (L̄) in parts 1 (A) and 4 (B) of the ET for both stents. The total area of the ET (ROI) was set as 100%.
Stent dimensions and lumen-tissue interface

In general, both implanted stent sizes were expanded up to their nominal diameter (Table 2). In part one, close to the nasopharyngeal opening, five of the six stents were expanded practically to their full diameter. In contrast to this, all six stents displayed a smaller degree of expansion in part four, at or close to the bony isthmus (Fig 7). The struts of both stents generally maintained their circular arrangement and could be detected in the sub-mucosal layer. However, in animal three, the struts in part 4 appeared to be deeper in the tissue, leaving the mucosal layer and being located already in muscle and gland tissue. The general number of struts lying free in the lumen ranged from 6.5% to 43% and is generally higher in the right ETs, e.g. the smaller stent (Fig 8).

The first mucosal layer on the interface between mucosa and lumen of the ET consisted in each ET of prismatic epithelium. The luminal side of this epithelium was covered with cilia, which could also be detected in the respiratory epithelium of the nasal cavity (Fig 9). In sheep three, both ETs showed moderate signs of mucosal detachment and autolysis, yet fragments of prismatic epithelium and cilia could as well be detected in both auditory tubes in this animal.

![Figure 7](image-url) Mean stent area of parts 1 and 4 of the implanted 2.75 mm x 26 mm (A) and 2.0 mm x 20 mm (B) stents with the calculated reference for each stent size. The data are shown as mean + SD.
3. STENTING THE EUSTACHIAN TUBE TO TREAT CHRONIC OTITIS MEDIA

![Graph showing percentage of struts not covered by tissue from nasopharynx to middle ear for both types of stents.](image)

**Figure 8** Percentage of struts not covered by tissue from nasopharynx (part 1) to middle ear (part 4) for both types of stents.

![Image showing interface between mucosa and lumen of the auditory tube (ET) and the nasal cavity (NC) of animal three (right ET, part 4), showing prismatic epithelium (Prism. epith.) topped with cilia at 2x magnification.](image)

**Figure 9** Interface between mucosa and lumen of the auditory tube (ET) and the nasal cavity (NC) of animal three (right ET, part 4), showing prismatic epithelium (Prism. epith.) topped with cilia at 2x magnification.

**Discussion**

Few therapeutic approaches utilize the Eustachian tube itself and the nasopharyngeal approach for the treatment of chronic otitis media and Eustachian tube dysfunction without surgical alteration of middle ear and tympanic membrane. Improvement of middle ear ventilation or transport of mucus via ventilation tubes in the eardrum causes eardrum perforation and creates new entrances for pathologic germs from the external auditory canal. If used repeatedly, atrophic
scarring of the tympanic membrane, myringo- and tympanosclerosis, eardrum retraction, persistent perforation or granulation tissue formation become apparent with an incident of 51% [25-26]. Stenting the auditory tube could improve ventilation of the middle ear and clearance of secretion through the ET itself, preventing eardrum perforation and in long term, middle ear destruction.

Thus, the aim of the present study was to develop the methodology of implantation *in vivo* and to evaluate the efficiency of an implanted bare metal coronary stent to maintain the patency of the Eustachian tube in blackface sheep. A second aim was to develop a model of induced aseptic otitis media with effusion, as the need for an intervention is only given when the function of ET is impaired.

The application of fluids, e.g. inflammatory mediators was easily feasible in the middle ear of sheep via the ET. Like the description for human practice [13], small amounts of fluid of one to two millilitres could be instilled in the sheep through the working channel of the flexible endoscope using a balloon catheter perforated at its tip. The amount of substances deposited in the middle ear remains unclear because of visible reflux from the nasopharyngeal orifice. This reflux might be promoted by pharyngeally directed cilium activity [27] which is needed by the ET for clearance from mucus produced in the lumen. However, a considerable amount of fluid, and therefore inflammatory mediators potentially reached middle ear and auditory tube undergoing the physiological absorption from middle ear and tubal mucosa. In contrast to the findings in the chinchilla model of induced otitis media with effusion triggered by inflammatory mediators applied through the bulla [21], no signs of a moderate or severe inflammation having a temporal connection to the instillation of platelet activating factor (PAF) and prostaglandin E$_2$ (PgE$_2$) could be observed. The effective dosage of PAF to induce middle ear effusion in mongrel dogs is reported to be between $10^{-7}$ mol/L and $10^{-6}$ mol/L [28], indicating that the first used dosage of $10^{-5}$ mol/L in the current study and especially the doubled concentration in the second application, should have been sufficient to trigger the desired effect. According to a study investigating the inflammatory potency of PAF, induced OME lasts for up to 14 days in chinchillas due to the initiation of an inflammatory cascade, although the initially instilled mediator was already cleared from the body [22]. However, the peak of inflammatory signs is suggested to be on day 4 post inflammation [21]. For these reasons we expected the inflammation in the sheep to peak during the first week after onset of the process, and to be visible still on day seven. Due to species restraints regarding the frequency of anaesthesia (limited to once a week), the follow up was performed on day seven after the instillation of PAF and
3. STENTING THE EUSTACHIAN TUBE TO TREAT CHRONIC OTITIS MEDIA

This possibly explains the absence of severe inflammatory signs in the endoscopic controls under GA, but the absence of moderate symptoms of inflammation and symptoms in the health score still remains unsettled.

Stent implantation in vivo could be proven to be as easily feasible as in the cadaver (compare [18]). All animals displayed good health condition during the experiment and the sporadic incidences of serous secretion in animal three may be explained by dust particles in hay and straw, irritating the mucous membrane. This phenomenon was already observed before stent implantation and in veterinary praxis it is common in a variety of species living in dusty environments.

Five of the six inserted stents stayed in the position as implanted in the ET. Fixation of the shorter stents only in the cartilaginous part of the ET was always successful and the fixation procedure of the larger stent by clamping it into the isthmus of the ET was successful in two of three cases. In one animal, the stent migrated partly into the direction of the nasopharynx, reaching into the nasal cavity. However, the stent stayed in this position for the entire rest of the experiment. According to these findings a fixation at the isthmus may not be required. This fact is important particularly because the clamping procedure entailed a narrower stent diameter in the vicinity of the bony isthmus and local ET distortion with further impaired opening as well as clearance function. This may have led to an aggravated mucus transport and resulted in the obstruction of the stents and finally the ET through secretion as it can be seen in all correctly positioned 26 mm stents on CT scans. The single dislocated stent of this size has a position closer to that of the 20 mm stents and showed similar ventilation with a smaller degree of narrowing compared to the 26 mm stents clamped in the isthmus.

In general, mild inflammation was detected in the endoscopic images of the ET orifice at the nasopharynx as well as moderate connective tissue encapsulation in the histologic slices. Additionally, rare signs of inflammation and minimal accumulation of secretion were seen in the middle ears. Considering, that the entrance of pathologic germs is limited to the pharynx and prevention of ascents to the middle ear is one of the main tasks of the ET [29], the function of germ protection appears to be maintained. However, dislocation should be avoided, because when the stent dislocates into the nasopharynx, having direct contact with bacterial flora in the nasal cavities, an increase of purulent secretion and inflammation seems to be the consequence. Further the extruded part of the stent is a continuous mechanical irritation during all epipharyngeal movements. Nonetheless, the middle ear itself appears to be as unaffected as in the other animals.
The tissue lumen ratio implies a free lumen for ventilation of about 20% in both ET, which is 2.35 mm$^2$ in the larger and 1.5 mm$^2$ in the smaller stent. The isthmus, the narrowest portion of ET, measures 1 mm in width x 3.5 mm in height in the blackface sheep [18] and 1 mm x 2 mm [4] in humans, implying that the detected free lumen could be enough to promote permanent ventilation as it is described for Rüdinger’s safety canal in the tubal cartilage, which measures only 0.4 to 0.5 mm [30]. In contrast a stent diameter of 1.5 mm was not sufficient to maintain transport of secretion [7], thus, the obstruction with secretion in the 2.75 mm stent might be caused by the narrowing of the stent diameter at the isthmus, explaining, why the dislocated larger stent did not show total obstruction.

Additionally, the movement of the auxiliary gap, i.e. the movable space below the safety canal, which opens only in process of swallowing or yawning, is hampered by the ingrown stent (Fig 4), limiting its opening diameter to the stent diameter. This part of the ET physiologically provides an opening diameter of up to 6-10 mm, securing the clearance [30]. This diameter is reduced by the ingrown stent to 2.75 mm or less.

In coronary vessels, an overgrowth of the vascular graft with intima is desired, to prevent thrombus formation and reform a smooth surface, ideally mimicking the original surface of the endothelium [31]. This overgrowth could be observed in each of the specimens in the current study, leaving only an average of 25.4% of the struts in the lumen. Furthermore, the appearance of ciliated epithelium and prismatic cells, alike the epithelium in the nasal cavities and usually as well in the ET, indicates a reparation of the mucosal layer traumatized by the insertion of the stent. Both ETs of sheep three showed moderate signs of mucosal detachment and autolysis, probably due to the procedure of fixation and staining. However, fragments of prismatic epithelium and cilia could also be detected in both auditory tubes in this animal, suggesting that the same findings as for the other animals apply for this animal. The meshes of the stent may allow the epithelium between the struts to reform on top of them. Thus, the material is incorporated and the stent fixed in this position. This phenomenon may on one hand facilitate the clearance, being indicated by the few secretion being left in the middle ears suggesting a consequent transport of secretion, but may on the other hand bear the danger of excessive growth of tissue, blocking the ET in the isthmus. Finally, explantation of the stent is not advisable because the surgical removal would be accompanied by the removal of the total mucosa and endangers the ET to coalesce in the process of healing.
Conclusion

Application of fluids into Eustachian tube and middle ear of blackface sheep was feasible, but the reaction on the inflammatory mediators was not as profound as expected.
The non-traumatizing and minimally invasive procedure of stenting the Eustachian tube was transferred from cadaver studies to *in vivo* application without complications. The stent was well tolerated by the sheep and promoted ventilation of the middle ear and clearance of the auditory tube. Regarding the design of the stent it seems to be sufficient to place it only in the cartilaginous part of the ET and the length should not exceed 20 mm.

Acknowledgement

The authors wish to thank Steffi Rausch (Cranio-Maxillo-Facial Surgery), Miriam Behrendt and Jasmin Bohlmann (Otolaryngology) for technical support.

Supplemental Material

**Table S1** Health score modified according to Otto 1998

<table>
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<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intake of food and water</strong></td>
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</tr>
<tr>
<td>usual food and water intake, usual rumination</td>
<td>0</td>
</tr>
<tr>
<td>only treats and moderate rumination</td>
<td>1</td>
</tr>
<tr>
<td>no feeding and water intake, no rumination</td>
<td>2</td>
</tr>
<tr>
<td><strong>Behaviour and facial expression</strong></td>
<td></td>
</tr>
<tr>
<td>Interested in surroundings, nibbles straw, head is carried upright and straight</td>
<td>0</td>
</tr>
<tr>
<td>Depressed, tired, sporadic moderate head tilt and /or head shaking</td>
<td>1</td>
</tr>
<tr>
<td>Flehming, absent staring, permanent head tilt and / or head shaking</td>
<td>2</td>
</tr>
<tr>
<td><strong>breathing frequency</strong></td>
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</tr>
<tr>
<td>Up to 20 breaths /min</td>
<td>0</td>
</tr>
<tr>
<td>25-30% increase from reference value</td>
<td>0,5</td>
</tr>
<tr>
<td>more than 50% increase from reference value</td>
<td>1</td>
</tr>
<tr>
<td><strong>Additional anomalies</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Increased body temperature, serous nasal discharge</td>
<td>1</td>
</tr>
<tr>
<td>Fever, purulent or bloody nasal discharge</td>
<td>2</td>
</tr>
<tr>
<td><strong>Maximal score</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
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References:


4. Overall Discussion

4.1 Concise Presentation of the Results

4.1.1 Tympanometry

Impedance audiometric measurements in conscious sheep were performed in order to evaluate their usefulness in regard to middle ear ventilation, eardrum integrity, hypermobile, atrophic or scarred eardrum, middle ear effusion and ET functions. In general, tympanometry in conscious sheep is feasible. Prior to the measurements, habituation to the measuring procedure and the examiner needed to be performed. Minor artefacts occurred, due to the specific anatomic characteristics of this species and the settings of the tympanometer used in the present study (226 Hz probe tone and pressure change between 500 to 600 daPa/s). The origin of these artefacts could predominantly be traced back to the shape and structure of the tympanic membrane, the resonance abilities of the middle ear and the hysteresis of the elastic parts of the external auditory canal during pressure change. When the frequency of 226 Hz, which is used for human ears having a smaller tympanic cavity, is applied, the ear of the sheep becomes mass-controlled instead of stiffness controlled, showing w-shaped notches (Figure 2.3). For these otherwise normal tympanograms the tympanogram type $A_N$ was introduced. The necessary long-lasting unobstructed EAC could be achieved by a cleaning procedure under general anaesthesia and subsequent periodic ear flushing in conscious sheep (Figure 2.4). The predominantly achieved tympanogram patterns were $A_N$ with 57.86% and type $A$ with 23.57%, displaying physiologic middle ear and Eustachian tube function and intact tympanic membrane. In one sheep a narrowed ear canal produced predominantly type B tympanograms but lacked pathologic findings, which could also be observed in recent tympanometric measurements in dogs. In addition, the body weight of the twelve sheep showed no correlation to the volume of the external auditory canal. Therefore, the size of the tip may be chosen according to the shape of the EAC. The obtained tympanometric results in the sheep indicate that the detection of eardrum perforations and atrophic or scarred tympanic membrane seems to be given. In addition, the outcome regarding middle ear pressure and therefore ventilation of the middle ear and the pressure equalising functioning of the ET, as well as middle ear effusion are also reliable.
However, the detection of ossicular chain disruption based on the measurement of the TW e.g. type E pattern in the tympanogram could be misleading if the artefact of the notch occurs. In addition, the probe of the device needs to be located in the mainly bony part of the EAC, in close proximity of the tympanic membrane to prevent hysteresis artefacts during pressure change. Reference values for type A and type \(A_N\) tympanograms were provided, which may serve as guidance to other researchers in the detection of pathologic processes with the presented method in the sheep (Table 2.2).

### 4.1.2 Stent Implantation

The non-traumatizing minimally invasive procedure of stenting the Eustachian tube of the sheep was proven to be feasible *in vivo*. The instillation of fluids (the inflammatory mediators) into the ET, was as well feasible but despite of the doubled concentration of the used mediators compared to concentrations used in chinchillas, no matching inflammatory findings could be detected having a temporal connection to the instillation. By means of an endoscopic score (Table 3.1, Figure 3.2), used to classify the appearance of the pharyngeal orifice of the ET, the reaction of the tissue in the temporal course of the experiment could be evaluated. The endoscopic score displayed no inflammatory reaction in three cases, mild inflammatory reaction in two cases and in one case moderate inflammatory reaction. This scored moderate inflammatory reaction was induced by the single stent, which migrated in direction of the nasopharynx and was visible in the pharyngeal orifice by approximately one sixth of its length. The two different sizes of the commercial bare metal coronary stent were inserted into the ET without complication and were visualised in the computed tomography scans (Figure 3.3) in all cases. The shorter stent stayed alike the longer stent in the ET during the entire experiment. The position of the inserted stent could be confirmed in the CT scans (Figure 4.1). The three short stents were located in the cartilaginous part of the ET, whereas two of the long stents reached into the isthmus and therefore the bony part of the auditory tube. Regarding the function of the stents, ventilation of the middle ear was apparent for each case and both stent sizes (Figure 3.3). The degree of obstruction and ventilation of the stents were visualized in the CT scans but quantified in the histologic analysis. The tissue formation, occurrence of secretion and free lumen were evaluated in alizarin red methylene blue stained \(33 \, \mu\text{m}\) thin slices (Figure 4.2). A free lumen of approximately 20% was detected in both stent sizes (Figure 3.6) and the obstructing material was defined as predominantly secretion.
Figure 4.1 CT image and schematic illustration of both stents *in situ*. Figure (B) shows the CT image of both stents *in situ*. Depicted are the 2.76 mm x 26 mm (L) and the 2.0 mm x 20 mm stent (R) inserted in the ET. Middle ear (ME), nasopharynx (NP) and spinal chord (SC) were labelled for orientation. To visualise the localisation, schematic illustrations of the short (A) and the long (C) stent are provided. External auditory canal (EAC), eardrum (ED), middle ear (ME), inner ear (IE), tympanic cavity (TC) and Eustachian tube (ET) with pharyngeal orifice (PO) and tympanic orifice (TO) are depicted.

An increase of tissue was accompanied by a decrease in lumen, secretion and free lumen from nasopharynx to middle ear. In addition, the reorganisation of ciliated epithelium (Figure 3.9) and the stent area (Figure 3.7) were evaluated. Incorporation of the struts into the mucosa of the Eustachian tube and reorganisation of prismatic epithelium topped with cilia was detected in each case. All six stents were generally expanded up to their nominal diameter (Table 2). In the proximity of the bony isthmus a smaller degree of expansion could be detected (Figure 3.7). Due to this narrowing, transport of secretion appeared to be hindered in the longer stent being fixed in the isthmus by clamping. Nonetheless, the incorporation of the struts and the reparation of prismatic and ciliated epithelium of the mucosa on top of the struts might as well provide clearance. However, this incorporation might bear the danger of excessive growth of tissue, obstructing the ET in the isthmus although this kind of obstruction could not be demonstrated in the sheep. The protection against pathologic germs appears to be maintained. The detected free lumen of 20% in both stents with a minimum of 1.5 mm² in each histologic slice, indicated a sufficient ventilation of the middle ear. However, the incorporation of the stent may hamper full opening of the ET, due to the limitation of the opening to the diameter of the used stent.
4. OVERALL DISCUSSION

Figure 4.2 Exemplary slice of the left ET of Sheep 1 (Chapter 3). Depicted are temporal bone (TB), tubal cartilage (TC), gland tissue (GT), mucosa of the ET (M), secretion in the lumen of ET (S) and the struts of the implanted stent (arrow) sized 2.75 mm x 26 mm.

4.2 Discussion

For the establishment of a valid treatment concept for chronic otitis media and chronic Eustachian tube dysfunction based on the stenting of the Eustachian tube, an adequate animal model and testing batteries for specific functional evaluation were not available yet. Rapid progress in material science as well as functionalisation of implants enable the development of novel devices for various applications. In order to overcome the shortcomings associated with research on the tuba Audita Eustachii, the present thesis aimed on the development of a minimally invasive surgical approach for the in vivo stenting of the Eustachian tube in a large animal model. In addition a method for the functional evaluation of the implanted device was established. The sheep was chosen as a large animal model because of the anatomical similarity to human Eustachian tube.

For the evaluation of a medical device, data in regard to the reaction of the animal model to the implant are needed to measure efficiency and compatibility of the product. Thus, appropriate methods needed to be developed and adapted to the middle ear and the Eustachian tube of the sheep. These methods include the minimally invasive stent application, induced
aseptic middle ear inflammation, evaluation of the in vivo reaction on the implant in the region of the pharyngeal orifice by means of an endoscopic score, tympanometry for the assessment of middle ear function and structures, computed tomography for the assessment of location of the stent as well as ventilation of middle ear and stent and histologic analysis of tissue formation, stent diameter and regeneration or reparation of prismatic and ciliated epithelium. In the following, advantages and disadvantages for each method focused on functionality and possible refinement are discussed. Not in detail described parts of the discussion can be found in the particular discussion of chapter 2 and 3. Functional evaluation of the tested coronary stent will not be discussed in this thesis and is provided in the discussion of chapter 3.

The application of the stent used in this study was performed via the nasopharyngeal endoscopic approach described in chapter 3. Former applied blind catheterisation of the ET had severe consequences like failed insertion into the nasal cavities or induced emphysema by application of substances under the mucosa. In the current study stent implantation was conducted under visual control via the endoscope. This procedure reduces treatment failures occurring during blind catheterisation to rare instances, which were not observed during the study presented in this thesis. In addition, both applied stent sizes fitted entirely into the ET of the sheep, which may suggest, that the opening of the pharyngeal orifice of the ET is facilitated but the orifice itself remains closed. Thus, the important physiologic function of protection against autophony and pathologic germs were maintained.

In a different study the chinchilla was used to test the biocompatibility and functionality of a poly-L-lactate stent. The stent, being applied via the bulla stayed with its proximal part in the ME and produced severe inflammation. The chinchilla provides smaller nostrils, ET and nasal cavities, making nasal approach to the ET difficult and hampering total insertion into the ET of stents shaped for human dimensions. Therefore, Litner et al. suggested using a different animal model with larger dimensions of the ET in order to use the stent design already adapted for human application. The testing of this device on functionality and biocompatibility would then be possible and limited to the ET, not altering ME structures. Additionally, the used transbulla approach implies the surgical opening of the ME and may induce inflammation and a prolonged healing process, since wound infections still are of clinical relevance despite antiseptic techniques. Thus, the nasal approach to the ET via the nostrils and nasal cavities in the sheep maintains the integrity of the middle ear and prevents complications associated with wound healing, due to the atraumatic proceeding. Furthermore, the sheep provides the required anatomy allowing the insertion of larger stent designs than those, the chinchilla model would allow.
In the present study, the used stent was already commercially available for a different indication. Thus, it was mounted on the balloon catheter used for application, which could easily be manoeuvred through the working canal of the endoscope. For the testing of different stents, which may have a larger diameter before implantation, the application through the working canal of the used endoscope may not be possible. In these cases an insertion tool could be used, similar to the already available design in the Bielefelder balloon dilatation or by using an endoscope with a larger working canal. Nonetheless, stent implantation with the here used device was feasible and may as well be adaptable to different designs of stents or other medical devices being applied in a similar way. Furthermore, the anatomy of the sheep allows the testing of stents in the dimensions for human application. In medical practice, the therapeutic implantation of stents into the ET would only be performed if an inflammation of the middle ear occurred. Thus the induction of aseptic middle ear inflammation was performed to mimic implantation into a diseased ET and ME.

The induction of aseptic otitis media with effusion in sheep, as described in chapter 3, was not as successful as expected. The restrains in the frequency of anaesthesia in sheep, limited to once a week, may have hindered the detection of the peak of inflammatory signs (Chapter 3). No health impairment or signs of OM like head tilt or head shaking were observed in conscious sheep after instillation of the inflammatory mediators applied under general anaesthesia. Additionally the narrow and angled EAC aggravates visualisation of the eardrum during consciousness, hampering examination of ET and eardrum in short (e.g. daily) intervals. Besides these limited examination possibilities, the precise quantity of fluid, which may have reached the middle ear, could not be evaluated. Due to the fact, that the sheep’s anatomy provides a remarkably larger tympanic cavity than humans the instilled amount of fluid (max. 2 mL) may not have reached the tympanic cavity in total. Therefore, the triggering process especially in the middle ear may not have been as profound as in the chinchilla. In the first attempt to trigger inflammation prior to the implantation of the stent, the concentration suggested by Ganbo et al. for the chinchilla was used. No inflammatory signs, neither in the clinical examination nor in the endoscopic examination in the following anaesthesia seven days after triggering could be detected. Thus, one week prior to the sacrifice inflammatory mediators were applied in doubled concentration having had as well not the expected effect. The time points of application were chosen to mimic the insertion of the stent into an inflamed ear and to evaluate the reaction of the stent onto swellings and the transport of accumulating secretion. Despite successful instillation of the fluid at both time points, the desired effect could
not be observed. Furthermore, Messenger and Papich 36 suggested, that application of carprofen, which is categorised as a non-steroidal-antiinflammatory drug NSAID, decreases the concentration of prostaglandine E$_2$ (PgE$_2$) in the interstitial fluid after triggering inflammation in dogs. This effect is based on the inhibition of the cyclooxygenase which is part of the inflammatory cascade 37. Therefore, carprofen has analgetic, antiinflammatory and antipyretic effects. The decrease of PgE$_2$ was also detected in a further study, examining the treatment of experimentally induced uveitis in dogs 38 but was not as profound as the reaction observed in the same study with other active substances. However, no references for any effect of carprofen on platelet activating factor (PAF), the additionally used inflammatory mediator, could be found in literature. Therefore, it may be left unaffected. In the current study postoperative pain management after general anaesthesia was performed with a single shot dosage of carprofen, which may have produced a decrease of PgE$_2$ concentration in the plasma but may not have affected the additionally applied PAF. Therefore, a milder inflammatory reaction with fewer symptoms of inflammation may have been the consequence, which could not be detected in the control on day seven. The total absence of inflammatory signs in the health score, however, remains unclear. Larger amounts of fluid as well as possibly higher concentration than those used by Ganbo et al. in the chinchilla and in the present study might be necessary to induce aseptic otitis media in the sheep. Furthermore, no cyclooxygenase inhibitor should be used as analgesic medication and a method to allow investigation of eardrum and pharyngeal orifice of the ET in shorter intervals may contribute to establish a model of induced aseptic otitis media in the sheep. Thus, further development of the used method described in chapter 3 or the development of a different method of induced inflammation may be needed.

The occurring inflammatory processes induced by the implanted stent during the course of the experiment were monitored by means of an endoscopic examination of the pharyngeal orifice of the ET. The endoscopic score, described in detail in chapter 3, was used to evaluate the inflammatory reaction, the appearance of secretion and the degree of opening of the nasopharyngeal orifice of the ET during the course of the experiment. In addition, an analysis of the visibility of the stent in the pharyngeal orifice was performed. The visibility and therefore the location of the stent in the pharyngeal orifice of the ET matches the findings obtained in the computed tomography (CT) scan and therefore described the location of the stent during the period of the experiment adequately. An estimation of the opening degree could also be performed in the endoscopic picture, reflecting the extent of possible immigration of pathologic germs from the nasal cavities into the ET. Additionally, the inflammation was evaluated using
redness and swelling, two of the cardinal symptoms of an inflammation firstly described by Aulus Celsus (✝ AD 38) and Galen of Pergamon (* AD 129). Pain, heat and impaired function could not be evaluated endoscopically and were therefore replaced by the quality of occurring secretion, scoring from none to purulent. These parameters could well be differentiated in the endoscopic picture (Figure 3.2), allowing a good description of the degree of inflammatory reaction of the organism onto the stent as well as the inflammatory situation in the pharyngeal orifice of the ET. Information about the actual degree of functioning in vivo could not be obtained by this method. Therefore, the method may benefit from a combination of functional testing, and endoscopic observation. This is already performed in humans by the combination of sonotubometry during the procedure of endoscopy. Unfortunately, endoscopy including sonotubometry requires a conscious patient for the act of swallowing, limiting this method to the application in humans. Thus, the endoscopic evaluation provides a reliable monitoring of the position of the stent in situ and the reaction e.g. the degree of inflammatory processes onto the implant. The data is valuable and can be obtained in vivo. Therefore, endoscopic evaluation allows the assessment of the implanted stent under general anaesthesia during the entire course of the experiment.

Since sonotubometry cannot be performed in sheep, impedance audiometry e.g. tympanometry was investigated on its practicability and reliability as method for the indirect functional testing of the ET. **Tympanometry** for the assessment of middle ear function has already been tested in a variety of other species. Despite minor artefacts, impedance audiometry is a useful non-invasive technique for the diagnosis of otitis media, the detection of perforated tympanic membrane, and for monitoring middle ear status in many species of experimental animals. It can provide information about the texture of the eardrum, eardrum perforation, ossicular chain integrity, ET function and middle ear ventilation. Comparable results to those of the sheep were received in two studies using the dog as animal model. Cole et al. performed the measurements under anaesthesia, and Strain & Fernandez provided data from mildly restrained conscious dogs. The value ranges of the sheep provided in chapter 2 differ from those described for sedated or conscious dogs. The results of the comparable study in sedated dogs suggest a similar ECV (mean: 0.94 mL) and a marginally larger TPP (mean: 53.94 daPa). The SA, however, is obviously smaller (mean: 0.47 mmho). In conscious dogs the ECV value (mean: 1.96 mL), exceeds those obtained in the sheep and in sedated dogs whereas the TPP (mean: 27.8 daPa) has a similar value as in sheep. The SA can be given in mL, which is comparable to the static admittance used in the sheep. However, the SA is analogue to those in sedated dogs and smaller than in the sheep. The difference in ECV may be due to the location of
the probe in the EAC as described in chapter 2. In both canine studies and in the conscious sheep the pressure at maximum compliance indicates an increased pressure in the middle ear. The values achieved in sedated dogs were highest. In conscious animals a marginally positive middle ear pressure appears to be a frequently occurring situation and should be taken into account for the interpretation of the tympanograms in these species. Speed of pressure change derived differences in the SA are reported as increase and shall be related to the hysteresis e.g. delayed adjustment of the elastic parts of the EAC to the pressure change. Thus, the difference in SA of the sheep from the mentioned studies in dogs might result from the speed of pressure change used (500-600 daPa/s). Slower change of speed (200 daPa/s or less) is recommended in human application. Unfortunately, information about this speed is not provided in both canine impedance audiometry studies. Considering the texture of the eardrum, eardrum perforation, ossicular chain integrity, ET function and middle ear ventilation, only the ossicular chain integrity could not be interpreted with sufficient certainty in the conscious sheep, due to occurring specific artefacts in the pattern of the tympanogram (Chapter 2). Thus, the measurements reflect an almost complete overview over the functions of the middle ear. A parameter, which may find consideration in following studies, is the stapedius reflex, which can be measured with the same handheld tympanometer being used in the current study. In dogs successful measurements were already performed under sedation and anaesthesia, suggesting that this method will also be feasible in the sheep. In regard to the functions of the auditory tube (Figure 1.3) only the ventilation of the middle ear could be tested. The clearance function can be interpreted indirectly in the occurrence of profound effusion in the middle ear. The protection against pathologic germs cannot be examined at all. In addition, the measurements reflect the status of ET and ME to a specific time point and give no long term information about the status. Thus, alike with Schilder et al., a combination of different methods appears to be needed. However, tympanometry in the sheep reflects almost the complete spectrum of ET functions and provides a good overview about the functioning of the middle ear and the ventilation via the ET, being one of the few procedures performable in conscious sheep.

However, post-mortem (indirect) investigation of ET functions (ME ventilation) could be performed by means of a computed tomography scan (CT). The CT scans performed in the present study (Chapter 3) allow the examination of the stent for its entire length in the ET. Furthermore, the ventilation of ME and stent could be assessed, as well as the occurrence of obstructive material in both structures. In a conventional radiologic image, however, superpositioning of bone and soft tissue hamper the visualization of the fine struts of the stent,
measuring only 60 µm in diameter. With the sectional imaging of the CT scans this problem could be solved. The detection of the cobalt-chrome stent was possible in 100% of the specimen in this study for its entire length. Ventilation, apparent by air in the lumen of stent and middle ear, was displayed and obstructions therefore detectable by increased opacity of the lumen. However, analysis of obstructing material is limited to the detection. The quality of these findings e.g. the differentiation between secretion and tissue, cannot be defined in this type of analysis. Furthermore, the scans were performed post-mortem, allowing no statement about the position of the stent directly after implantation several weeks before sacrifice and in the temporal course of the study. Therefore, periodically applied CT scans would be beneficial for the monitoring of stent position and degree of obstruction during the experiment. In regard to the material of different implants, opacity may differ and therefore the method of imaging may need to be adjusted to the used material. Consequently, CT scans delivered profound information about location, ventilation and obstruction of the bare metal cobalt chrome alloy stent as well as ventilation and appearance of effusion and/or soft tissue in the ME.

To discriminate between accumulation of secretion and growth of tissue in the lumen of the stent the CT scans need to be complemented by a method allowing this distinction. Therefore, a histologic analysis of tissue formation, stent diameter and the occurrence of prismatic and ciliated epithelium was performed (Chapter 3). To analyse the obstructing material seen in the CT scans and to evaluate the proliferative reaction on the stent, as well as the degree of free lumen, warranting the ventilation of the middle ear, tissue formation was analysed. For this purpose four representative parts along the course of the ET were defined and three consecutive 33 µm thin histologic slices were analysed according to the parameters mentioned above (detailed description provided in Chapter 3). The analysis followed the different parts of the ET and could therefore detect differences in the reaction of the ET on the stent in the different anatomic surroundings (e.g. mainly cartilaginous versus mainly bony part of ET). However, limiting the analysis to four sections, isolated from each other, the effect of the stent onto the ET between these four parts remains unconsidered. In addition, no analysis was performed in the sections of the ET being located proximal and distal from the stent. Thus, the tympanic orifice and not stented parts of the ET were not examined.

The quality of obstructing material e.g. the differentiation between secretion and the growth of proliferative tissue as well as the amount of free lumen and incorporation of the struts could be assessed. To verify if the transverse section of the stent has the same area as the fully
expanded not implanted stent, an analysis of the **stent diameter**, e.g. the area between the struts was performed. These data were compared to the calculated reference from the manufacturer. By this method, the degree of expansion could be detected. Total collapse of the stent was defined by the inability to fit the ellipse (detailed description in Chapter 3) onto or close to the struts of the stent. Collapse might be a result of the manipulation during preparation or the compression of the ET caused by the specific muscles during physiologic function and was not observed in the analysed slices. To verify these results and rule out cases, where the stent may still provide a similar area between the struts alike the reference analysis of the two diameter of the ellipse could be beneficial. Is one diameter prominently shorter compression of the stent or oblique-cut of the specimen may be the case and need to be considered in the interpretation.

Additionally to this analysis, the interface between lumen and tissue of the ET was evaluated regarding **prismatic and ciliated epithelium**. In the used 40x magnification both, prismatic epithelium and cilia could be detected. The Alizarin red and Methylene blue staining allowed good differentiation between bone (red), cartilage (violet) and soft tissue (different shadings of blue) (Figure 4.2).

The prismatic cells could be differentiated according to their specific appearance and their nucleus (Figure 3.9). The cilia on top of the prismatic cells displayed a brush like appearance and could well be distinguished from other cells or artefacts occurring in lumen and secretion. A beneficial additional information to the histology may include functional analysis of the ciliated epithelium. A measurement could include transport of secretion in vivo. In addition to the endoscopic investigation of inflammatory reaction, analysis of the cellular reaction in direct proximity of the struts may give evidence of the degree of foreign body reaction to the implanted material.

Generally, the histologic analysis was performable with the used specimen preparation and staining procedures. The analysis of ROI and ratio of tissue, secretion, lumen and free lumen (Chapter 3) may as well be performable if no stent was implanted, for example if one ET is stented and the contralateral ET serves as control. This allows highly comparable results between treated and untreated groups because the methods of analysis resemble each other to a very high extend.
Since 2015 the recommended combination of tests for the assessment of OM and especially of ET functions include otoscopy, otomicroscopy of the eardrum, tympanometry, Rinne’s and Weber’s tuning fork test, pure tone audiometry and nasopharyngoscopy of the opening of the ET. For the tuning fork tests and the pure tone audiometry active contributing to the testing is needed and difficult to obtain in the sheep. However, in this thesis it could be proven that the method of tympanometry can be performed in conscious sheep. Otomicroscopy and otoscopy could be substituted by an endoscopic examination of EAC and eardrum combined with the nasopharyngoscopy, which could be performed under general anaesthesia. To gain deeper insight into tissue reaction, stent expansion and the specific location of the used stent, the test-series was extended by CT scans and histologic analysis.
5. Conclusion & Outlook

The obtained findings provided sustained scientific statements in regard to the compatibility and tolerance of the stenting material, as well as the degree and quality of obstruction and the stability of the used stent. The combination of tests provided a diverse spectrum of data, facilitating the evaluation of the studied stent. Even though the temporal follow up of the implant could be improved by additional CT scans, performed \textit{in vivo}, in general, the methods were well suitable to provide the necessary data to evaluate the stent used in the current study. The discussed refinements of each method may improve the collected data. Still a profound series of tests could be made available for the evaluation of stents and similar implants in the ET.

Further research could focus on the refinement of the presented methods as well as the extension of the testing series on hearing abilities of the sheep, temporal follow-up during the experiments and methods for the evaluation of degradation of different materials. Inflammatory processes on cellular level may improve histologic analysis. Furthermore, the method of induced aseptic otitis media has to be improved or needs to be replaced by a more suitable method for the sheep.

Thus, it can be inferred that the sheep as large animal model for stenting the ET is as well suitable \textit{in vivo}. Additionally, the here presented methods of the assessment of stents in the sheep provide a profound basis for the testing of these kinds of medical devices in pre-clinical studies.
References:


AFFIDAVIT

I herewith declare that I autonomously carried out the PhD-thesis entitled

“Stenting of the Tuba Auditiva as a Valid Treatment Concept in Chronic Otitis Media and Chronic Tube Dysfunction and its Sequeleae: Implementation of a Large Animal Model”

No third party assistance has been used.

I did not receive any assistance in return for payment by consulting agencies or any other person.

No one received any kind of payment for direct or indirect assistance in correlation to the content of the submitted thesis.

I conducted the project at the following Institution: Department for Laryngology, Rhinology and Otology, Hannover Medical School

The Thesis has not been submitted elsewhere for an exam, as thesis or for evaluation in a similar context.

I hereby affirm the above statements to be complete and true to the best of my knowledge.

____________________________
Date, Friederike Pohl
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Love to all of you…