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# THE EFFECT OF ALBUMIN COATING ON PROPERTIES OF TYMPANOSTOMY TUBES

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ACADEMIC DISSERTATION
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Cover: Scanning electron microscope images of an extruded uncoated titanium tympanostomy tube covered with biofilm (magnification x30, x100, x500, x5000).

*To my family*

# ABSTRACT

Otorrhea and early tympanostomy tube (TT) occlusion represent a major problem in the outcome of patients after TT insertion. Because albumin coating of implant devices can prevent adhesion of platelets, proteins, and bacteria, this study investigated whether TTs coated with albumin are more resistant to typical TT sequelae than are uncoated tubes.

Three studies with albumin-coated TTs and one study with titanium implants were conducted: The binding of fibronectin, one of the most adhesive proteins known, on these TTs was tested first (I). Next, to learn whether the albumin coating is durable on the implant surface, an 8-month trial tested the adhesion of fibronectin to the TT surface after storage of the tubes for 1, 3, and 8 months in three environments (II). Adhesion was then tested of the two most typical bacteria causing TT otorrhea, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, to albumin-coated and -uncoated titanium implant plates (III). After these *in vitro* experiments, knowing that the albumin-coated surface is repellent to both bacterial and protein adhesion, the effectiveness of the method was tested in a prospective clinical study (IV). In this prospective randomized double-blind follow-up, the 170 patients enrolled suffered from otitis media with effusion (OME) or recurrent acute otitis media (RAOM). To discover the difference in frequency of sequelae in albumin-coated and uncoated tubes, one of each type (total : two) was inserted into each patient.

The results indicated that the albumin-coated surface was resistant to protein adhesion (I) and bacterial adherence (III), and that the capacity to prevent adhesion of this protein endured for the entire 8-month trial (II). In the prospective trial (IV), incidence of typical sequelae of tympanostomy, otorrhea and early tube occlusion, in the albumin-coated tubes were diminished. Early tube occlusion was half as frequent in the ears with albumin-coated tubes (IV). Especially in patients with perioperative bleeding was this difference marked.

An implant coated with albumin is thus resistant to protein and bacterial adhesion *in vitro*, and the coating is durable. Albumin coating of TTs can provide *in vivo* an effective method to limit the number of TT sequelae.

# TIIVISTELMÄ

Ilmastointiputken asettaminen tärykalvoon on yksi yleisimmistä lapsille tehtävistä toimenpiteistä. Putken kautta tuleva märkävuoto ja ilmastointiputken ennenaikainen tukkeutuminen ovat merkittävimmät kyseisen hoidon tehoa uhkaavista ongelmista. Koska muissa elimistöön asetettavissa materiaaleissa on voitu todeta, että albumiinipinnoitus vähentää bakteerien ja verihiutaleiden tarttumista materiaalin pintaan, halusimme selvittää vähentääkö albumiinipinnoitus edellä mainittuja ongelmia tärykalvon ilmastointiputkissa.

Neljässä erillisessä tutkimusessa selvitettiin humaani seerumin albumiini (HSA) -pinnoituksen vaikutusta tärykalvon ilmastointiputkien ja titaanilevyjen ominaisuuksiin: ensimmäisessä tutkimuksessa selvitettiin yleisimmän elimistön liimaproteiinin, fibronektiinin, tarttuvuutta albumiinipinnoitettuun ja pinnoittamattomaan ilmastointiputkeen (I). Toisessa tutkimuksessa selvitettiin albumiinipinnoituksen pysyvyyttä. Pinnoitettuja ja pinnoittamattomia ilmastointiputkia säilytettiin lämmön ja ilmankosteuden suhteen erilaisissa ympäristöissä 1, 3 ja 8 kuukauden ajan, jonka jälkeen testattiin fibronektiinin tarttuvuus putkiin (II). Kolmannessa tutkimuksessa selvitettiin tyypillisimpien putkivuotoa aiheuttavien bakteerien, *Staphylococcus aureuksen* ja *Pseudomonas aeruginosan*, tarttuvuus albumiinipinnoitettuun ja pinnoittamattomaan titaanilevyyn (III). Koska edellä mainituissa tutkimuksissa selvisi, että albumiinipinnoitettu materiaali hylkii sekä bakteereja että proteiineja, teimme prospektiivisen, satunnaistetun potilastutkimuksen albumiinipinnoituksen kliinisen tehon selvittämiseksi (IV). Kyseessä oli kaksoissokkoutettu seurantatutkimus, johon otettiin 170 potilasta. Potilaat tulivat tärykalvojen putkitukseen joko liimakorvataudin tai toistuvien akuuttien välikorvatulehdusten vuoksi. Jokainen potilas sai toiseen korvaan pinnoitetun ja toiseen pinnoittamattoman titaaniputken.

Tulokset osoittavat, että albumiinipinnoitus vähentää liimaproteiinin (I) ja bakteerien tarttuvuutta (III), ja että pinnoituksen aikaansaama liimaproteiinin tarttuvuutta hylkivä ominaisuus säilyy pinnoitetussa putkessa 8 kuukauden varastoinnin aikana (II). Prospektiivisessa tutkimuksessa ilmeni, että tyypillisimpien putkituksen tehoa uhkaavien ongelmien määrä oli pienempi albumiinipinnoitetuissa putkissa (IV). Verrattuna pinnoittamattomaan titaaniputkeen, kolmen ensimmäisen seurantakuukauden aikana putkitukoksia todettiin 50 % vähemmän albumiinipinnoitetuissa putkissa. Erityisen merkittävä ero oli potilailla, joiden korvissa ilmeni verenvuotoa putkituksen yhteydessä.

Ilmastointiputken pinnoitus albumiinilla vähentää proteiinien ja bakteerien tarttuvuutta *in vitro* ja pinnoituksen tuottama tarttuvuutta vähentävä ominaisuus on pitkäkestoinen. Tärykalvon ilmastointiputkien pinnoittaminen albumiinilla tarjoaa käytännössä tehokkaan tavan rajoittaa putkissa ilmenevien ongelmien määrää.

# CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| **AgO** | Silver oxide |
| **AOM** | Acute otitis media |
| **CFU** | Colony-forming unit |
| **ECV** | Ear-canal volume |
| **HSA** | Human serum albumin |
| **MEE** | Middle ear effusion |
| **OME** | Otitis media with effusion |
| **PBS** | Phosphate-buffered saline |
| **RAOM** | Recurrent acute otitis media |
| **SEM** | Scanning electron microscopy |
| **TT** | Tympanostomy tube |

# ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I to IV.

**I** **T.J. Kinnari, E-M. Salonen, J. Jero.** New method for coating tympanostomy tubes to prevent tube occlusions. *Int. J. Pediatr. Otorhinolaryngol.* 2001;58:107-111.

**II** **T.J. Kinnari, E-M. Salonen, J. Jero.** Durability of the binding inhibition of albumin coating on tympanostomy tubes. *Int. J. Pediatr. Otorhinolaryngol.* 2003;67:157-164.

**III** **T.J. Kinnari, L.I. Peltonen, P. Kuusela, J. Kivilahti, M. Könönen, J. Jero.** Bacterial adherence to titanium surface coated with human serum albumin. *Otology & Neurotology,* in press.

**IV** **T.J. Kinnari, H. Rihkanen, T. Laine, E-M. Salonen, J. Jero.** Albumin-coated tympanostomy tubes: Prospective, double-blind clinical study. *Laryngoscope,* in press.

# 1
# INTRODUCTION

Otitis media is one of the most usual childhood diseases. In children suffering either from recurrent acute otitis media (RAOM) or otitis media with effusion (OME) unresponsive to nonsurgical treatment, tympanostomy (myringotomy with TT insertion) has become the treatment of choice (Bluestone and Klein 1995). In the operation, a tube usually of silicone or titanium is implanted into an incision through the tympanic membrane. Tympanostomy is one of the most common operations on children in the industrialized countries. In the USA, where 2 million tympanostomy tubes (TT) are manufactured annually (Isaacson and Rosenfeld 1996), it is the most common pediatric procedure under general anesthesia (Derkay 1993). A TT is expected to bypass the malfunctioning eustachian tube and to secure middle ear ventilation so that the middle ear mucosa can heal by itself. The most typical sequelae with TTs are otorrhea through the tube and tube occlusion before its expected extrusion time (Kay et al. 2001). These problems are associated with contamination of the tube by exudates and bacteria (Karlan et al. 1981).

Many different methods to reduce TT sequelae have been tested: both systemic and local peri- and post-operative medications and ear canal treatments of different magnitude. Hundreds of tube models and materials exist, as well as tube coatings. Experiments show that tube model and material each play a role in the incidence of tube sequelae. For instance, silver oxide (AgO) coating on TTs diminishes the incidence of otorrhea (Chole and Hubbel 1995). Tube ionization has also shown potential in preventing tube contamination (Biedlingmaier et al. 1998).

Human serum albumin (HSA) has served as a plasma expander since World War II. The antiadherent characteristics of an HSA coating were already reported by Packham and colleagues in 1967. Since the 1970s, the HSA coating has served in extracorporeal hemoperfusion systems to prevent platelet adhesion and the subsequent blood clot formation on artificial surfaces of hemoperfusion devices. The HSA coating is also helpful in porous arterial graft prostheses to prevent bleeding through the prostheses during surgery. In the last decade, interest in the possibility to use the antiadherent characteristics of the HSA coating against peri- and postoperative bacterial contamination of implants has grown. Several *in vitro* and *in vivo* tests with HSA-coated implants (Lubin et al. 1986, Kottke-Marchant et al. 1989, Zdanowski et al. 1993, McDowell et al. 1995, An et al. 1996) have shown in all cases that the HSA coating provides an effective weapon against unwanted adhesion on and contamination of implants.

The research project presented here was conducted to study the effect of albumin coating on the properties of TTs both in laboratory surroundings and in real life. Until now, few if any prospective randomized clinical trials have examined HSA-coated implanted devices.

# 2
# REVIEW OF THE LITERATURE

## 2.1 OTITIS MEDIA

### 2.1.1 DEFINITION

Acute Otitis Media (AOM) is a clinically identifiable infection of the middle ear with acute onset of signs and symptoms and short duration (Klein et al. 1989). The appearance of the tympanic membrane is changed in AOM, and fluid is present in the middle ear (Puhakka et al. 1999). The nonspecific signs associated with AOM are fever, irritability, headache, apathy, anorexia, vomiting, and diarrhea. The most typical specific signs and symptoms are otalgia, otorrhea, hearing loss, and rarely vertigo, nystagmus, tinnitus, swelling of the ear, facial paralysis, and purulent conjunctivitis. AOM can occur with all or without any of these symptoms, and none of them, except purulent otorrhea, is reliable for diagnosis (Bluestone and Klein 1995).

Otitis media with effusion (OME) is a noninfectious condition without acute signs and symptoms with middle ear effusion (MEE) behind an intact tympanic membrane (Klein et al. 1989). Usually, after AOM, if MEE poorly resolves, it results in OME (Klein et al. 1989).

### 2.1.2 EPIDEMIOLOGY

Among pediatric patients, AOM is an extremely common disease. During the first 2 years of life, 70% to 91% of children experience at least one episode of AOM (Teele et al. 1989, Paradise et al. 1997), and some become "otitis-prone," with six or more episodes of AOM before the age of 6 (Howie et al. 1975, Faden et al. 1998). AOM accounts for 20 to 40% of all office visits for children under 6 in the USA (Teele et al. 1983). Furthermore, it is the most common cause of hearing loss during childhood (Teele et al. 1990). The incidence of otitis media is highest in spring and autumn, increasing close to the end of the first year of life and decreasing during the second year (Alho et al. 1991). Despite all efforts, the incidence of AOM and subsequent OME is rising. (Lanphear et al. 1997, Joki-Erkkilä et al. 1998). In Finland, cases of pediatric AOM diagnosed annually amount to half a million (Niemelä et al. 1999).

Incidence of OME is also dependent on age and time of year, being the highest in winter moths and during the second year of life (Bluestone and Klein 1995). In healthy children from 2 to 4, incidence of OME varies between 14.6% and 18.1% (Tos and Poulsen 1979, Tos et al. 1982). Spontaneous recovery from OME is very common, especially in the summer months: Between May and August, resolution of MEE can occur in up to 66% of cases (Tos and Poulsen 1979).

Because AOM is such a common disease, it causes marked financial losses both to the families and to the community. In the end of the 1990's, total annual cost of treating otitis media in Finland was an estimated € 115 million, the average cost of each attack of AOM was € 189, and the average annual cost arising from otitis media per child under age 2, with all costs such as visits to physicians, medication, parents' absence from work, and cost of surgery included, were € 863 (Niemelä et al.

1999). In the United States in 1997 the average cost of a simple episode of AOM was € 203 and of a complex case, with several visits and possible hospitalization, was € 607 (Capra et al. 2000).

### 2.1.3 ETIOLOGY AND PATHOGENESIS

The pathogenesis of otitis media is a complex combination of factors such as viral or bacterial infection, eustachian tube dysfunction, allergy, and the poor status of the immunosystem. Furthermore, environmental and social status contributes to its incidence (Paradise et al. 1997). The major risk factors associated with AOM are male sex, sibling history of AOM, early occurrence of first attack of AOM, and lack of breast feeding (Teele et al. 1989). In children, immature function of the eustachian tube (Bluestone et al. 1972) with ascending viral or bacterial pathogens of the nasopharynx (Rosenfeld 1996) seems to be the main factor promoting otitis media. The most usual bacteria involved are *Streptococcus pneumoniae, Haemophilus influenzae,* and *Branhamella catarrhalis* (Bluestone et al. 1992). Evidence of viral RNA has been detected in as high as 48% of AOM episodes (Pitkäranta et al. 1998).

The pathogenesis of OME is complex, as well. OME is diagnosed when MEE occurs in an asymptomatic ear. OME usually occurs after AOM with poor resolution of the MEE but can also arise spontaneously without pre-existing AOM symptoms (Rosenfeld 1996). Of the bacteria causing AOM, *H. influenzae* is isolated most frequently from patients with OME (Bluestone et al. 1992). Drainage from the MEE is blocked by the inflammatory swelling of the mucosa and impaired ciliary function in the middle ear which are mediated by cytokines and inflammatory mediators (Ganbo et al. 1995). In OME, the number of middle ear mucosal secretory cells increases, leading to formation of a mucin-rich effusion in the middle ear (Tos 1980, Carrie et al. 1992). Resolution of MEE is impaired more frequently in children because of their immature eustachian tube anatomy (Bluestone and Klein 1995). Recently, during AOM and OME, biofilm has been found covering the middle ear mucosal surface (Post 2001, Fergie et al. 2004). Evidence supporting the role of biofilm formation in the pathogenesis of AOM and OME may explain the fact that in OME, bacterial cultures often show no pathogens (Qvarnberg et al. 1990).

### 2.1.4 CLINICAL COURSE

Often AOM heals even without any special treatment in a few weeks (Burke et al. 1991), but prolonged and recurrent cases of otitis media are not rare (Pukander et al. 1982, Rosenfeld et al. 1994, Jero et al. 1997). During AOM, fluid is produced in the middle ear. Usually this fluid gradually disappears during the one to four-week healing process (Teele et al. 1980), but sometimes the inflammation continues without symptoms because of increasing obstruction of the eustachian tube, leading to failed middle ear clearance and retention of fluid in the middle ear, OME (Shurin et al. 1979).

In OME, the middle ear fluid can be either serous, mucoid or purulent (Tos 1980). Spontaneous resolution of the MEE is also usual in OME. Incidence of MEE one month after the first diagnosis of OME is 33%, after 2 months 20%, and after 2 years 2% (Thomsen and Tos 1981, Casselbrant et al. 1985). On the other hand, in patients with bilateral OME continuing for at least 3 months, likelihood of spontaneous resolution of the MEE is worse. The incidence of MEE in this group after 6 months has been 80%, after one year 78%, and after 3 years 51% (Maw and Bawden 1994 a).

### 2.1.5 COMPLICATIONS

The most usual complication after AOM and OME is hearing loss, which occurs to some extent in all ears with effusion (Teele et al. 1990). Fluid production in the middle ear gradually impairs the movement of the tympanic membrane, which causes conductive hearing loss (Bluestone and Klein 1995). Such hearing loss caused by untreated OME or recurrent AOM can lead to retardation of language skills and attention (Bluestone et al. 1983). In a group of children with cleft palate and OME treated with early TT placement, hearing acuity and consonant articulation was significantly less impaired than in children who had undergone myringotomy later (Hubbard et al. 1985). Several episodes of AOM in early life, even if effectively treated, can harm reading comprehension as late as at 9 years of age (Luotonen et al. 1996).

The more severe complications of AOM with inflammation spread intratemporally in areas such as mastoid cells, the petrous portion of the temporal bone, or inner ear, causing mastoiditis, facial paralysis, labyrinthitis, or sensorineural hearing loss, or intracranially, causing meningitis, brain abscess, or lateral sinus thrombosis, are rare (Bluestone and Klein 1995, Goldstein et al. 1998, Bluestone 2000). At present, less than 0.5% of the cases of AOM are associated with serious complications, compared with an incidence before the antibiotic era of over 40% (van Buchem et al. 1985).

### 2.1.6 DIAGNOSIS AND TREATMENT

Diagnosis of AOM and OME is based on medical history, the typical signs and symptoms listed above, and physical examination. The examination includes a general examination and pneumatic otoscopy (Puhakka et al. 1999). Because of difficulties often found in identifying an ear with effusion, either tympanometry or otomicroscopy is often recommended to confirm the diagnosis, especially when OME is suspected (Cantekin et al. 1980, Palmu et al. 1999).

Although spontaneous resolution of AOM is usual, antibiotic treatment is recommended (Rosenfeld 1996, Puhakka et al. 1999). Because of the high incidence of AOM, concern is growing as to overuse of antibiotics in its treatment. In the USA, over one-quarter of all oral antimicrobial agents are prescribed for AOM (Bluestone 1998). It has been estimated that for every seven children with AOM treated with antibiotics, only one really benefits (Rosenfeld et al. 1994). Based on the current consensus in treating AOM in Finland, antibiotic treatment with efficacy against the three major bacterial pathogens in AOM is recommended, but in selected cases watchful waiting with pneumatic otoscopy for 1 to 2 days after the first diagnosis of AOM is acceptable (Puhakka et al. 1999). Symptoms of AOM can also be relieved with analgesic medication. For recurrent attacks of AOM—three or more episodes during the preceding 6 months—TT insertion can be helpful (Bluestone and Klein 1995).

The effect of artificial middle ear drainage and aeration with a tympanic membrane incision in curing AOM was first described by the English surgeon Sir Astley Cooper (Alberti 1974), who planted the seed for the growth of tradition of myringotomy 200 years ago. Despite the traditional rule of surgery, "ubi pus ibi evaqua," myringotomy in treating AOM virtually ceased during the era of antibiotic treatment begun in the 1940s. In recent decades, the procedure has been limited only to those children with severe otalgia and suppurative complications such as mastoiditis (Bluestone and Klein 1995). In part, this is due to the dramatic increase in tympanostomy procedures during the last 3

decades (Black 1984), to the fact that incidence of the fearsome complications of AOM has been low (van Buchem et al. 1985), and to the fact that the relief of hearing loss and pain in AOM achieved with myringotomy is brief because the middle ear can rapidly again fill with fluid after the procedure (Berger 1989).

OME found in a symptomless patient is most likely due to an extension of AOM and usually resolves spontaneously (Bluestone and Klein 1995). Systemic steroid treatment has some limited effect in facilitating the spontaneous resolution of the MEE (Rosenfeld et al. 1991). In cases with MEE persisting over 3 months or unresponsive to medical treatment or in cases with shorter-length but severe conductive hearing loss, treatment with TTs is indicated (Bluestone and Klein 1995).

Adenoidectomy has been widely used as a treatment for RAOM or OME. Recent investigations have, however, shown that adenoidectomy, as the primary method of prophylaxis, has little effect on further episodes of AOM in children under 2, the age-group in which the incidence of AOM is highest (Koivunen et al. 2004).

## 2.2 TYMPANOSTOMY

### 2.2.1 TYMPANOSTOMY OPERATION

Placement of a tube in the myringotomy incision was suggested first by Adam Polizer in the 1860's (Alberti 1974). Not until Armstrong reported his favorable results in treating five patients with this method 50 years ago, in June 1954, did the method become popular. After the late 70´s TT placement has become the most common surgical procedure among children in many western countries such as the USA, the Netherlands, and England (Black 1984). In Finland, tympanostomy is performed on 10% of all children before the age of 5 years (Niemelä et al. 1999) and in the Netherlands, 2% of all children younger than 12 years receive TTs yearly (Schilder et al. 2004). In the USA, the number of TTs manufactured yearly is about 2 million (Isaacson and Rosenfeld 1996), and they are inserted annually into more than a million children (Mandel et al. 1984), which makes this the most common pediatric procedure performed under general anesthesia there (Derkay 1993). Because the incidence of AOM and OME is highest in children under 2, the majority of TTs are inserted in this age-group (Myer and France 1997). The surgical treatment of OME with TTs has been found cost-effective when recurrent OME fails to respond to medical therapy (Gates 1996).

In the operation, an incision is made into the anterior part of the tympanic membrane, thus protecting the ossicular chain located behind the posterosuperior part and the round window membrane located behind the posteroinferior part of the tympanic membrane. Fluid, if present, is routinely aspirated from the middle ear before the tube, usually with wide flanges at both ends, is inserted in the incision made in the tympanic membrane. The open tube allows the fluid to drain out and the ventilation of the middle ear, and restores hearing. The effect of TT treatment for bilateral OME lasting 3 months and unresponsive to nonsurgical treatment has been convincingly shown in several prospective studies (Gates et al. 1987, Mandel et al. 1989 and 1992). Furthermore, in treating AOM, the efficacy of TT has been evident among patients suffering from frequently recurring attacks of AOM three or more times during the preceding 6 months, along with failure in chemoprophylaxis (Gebhart 1981, Gonzalez et al. 1986, Casselbrant et al. 1992, Giebink 1994).

Normally the TT stays patent in the tympanic membrane from 6 to 18 months,

depending on the model and material of the tube (van Baarle and Wentges 1975, Handler et al. 1986 and 1988). The most common collar-button style tube, with wide flanges set at right angles to the lumen of the tube, stays in place about twice as long as the Shepard style tube with its shallow curve at the neck of the tube and minimal flanges (Moore 1990). Furthermore, shorter-range middle ear ventilation without tubes is available with laser-assisted myringotomy, although more relapses occur with this method (Silverstein et al. 1996). While the artificial middle ear ventilation is functioning, water-entry to the middle ear through the tube may lead to complications. Although the routine use of earplugs while bathing or swimming is not indicated unless the child has had frequent bouts of otorrhea, submersion in soapy water or deep submersion in any water increases risk for middle ear contamination (Pashley and Scholl 1984, Hebert et al. 1998).

The TTs migrate posteriorly around the umbo with the migrating epithelial layer of the tympanic membrane and finally extrude from the tympanic membrane, pushed out by the squamous debris which over time accumulates under the outer flange of the tube (van Baarle and Wentges 1975, Isaacson and Rosenfeld 1996). Surgical removal of the tube is required in cases where the tube, usually for an unknown reason, does not extrude spontaneously. Watchful waiting for at least 2 years after the tympanostomy procedure is recommended before any such procedures are done, if the tube is functioning normally, due to the risk for persistent perforation after tube removal (Cunningham et al. 1993, El-Bitar et al. 2002).

A child with TTs needs careful follow-up at 4- to 6-month intervals and at least one follow-up visit 6 to 12 months after tube extrusion for early detection of any possible TT sequelae (Rosenfeld and Bluestone 1999, Kokko 1974). Approximately 26% of tympanostomized patients will require re-tympanostomy after tube extrusion (Casselbrant et al. 1992). One explanation is continuing eustachian tube dysfunction. The effect of adjuvant adenoidectomy, tonsillectomy, or both on outcome of tympanostomized children is currently under active debate (Paradise et al. 1990, Coyte et al. 2001, Mattila et al. 2003). During the present era of antibiotic-resistant bacteria, the role of surgical procedures in treating such overwhelmingly common diseases as recurrent AOM and OME has become even more important (Bluestone 1998).

### 2.2.2 SEQUELAE OF TYMPANOSTOMY

Although TT insertion is a widely accepted, effective treatment for curing the conductive hearing loss in OME (Shah 1971), and it has been shown to improve the patient's quality of life (Facione 1991, Urben and Nichols 1996, Hellier et al. 1997), some sequelae can arise. The main problems interfering with TT function are early occlusion of the tube and inflammation of the middle ear with a purulent discharge, otorrhea (Kay et al. 2001). The tube becomes occluded in as many as 36% of the cases (Reid et al. 1988, Weigel et al. 1989). Usually the plug occluding the tube consists of dried mucoid effusion (Westine et al. 2002 a). Tested perioperative methods to reduce the incidence of tube occlusion, include coating the tube or tympanic membrane with vasoconstrictor solution (Altman et al. 1988, Jamal 1995) or Polysporin ophthalmic ointment (Cunningham and Harley 1992), or using prophylactic antibiotic/steroid ear-drops (Salam and Cable 1993). When a TT is occluded, one can try to solve the occlusion with ear drops (Brenman et al. 1986, Spraggs et al. 1995, Westine et al. 2002 b), or clear the blocked tube

mechanically with suction and irrigation. Clearance of the occluding plug may depend on tube surface properties (Tsao et al. 2003). In practice, methods to open an occluded TT are difficult and painful to carry out and usually not successful without general anesthesia and tube removal (Rosenfeld and Bluestone 1999). Almost one-third of tube occlusions lead to tube replacement (Per-Lee 1981).

In 17% of the intubated ears, the treatment is associated with otorrhea (Kay et al. 2001) which in OME is the most common complication of tympanostomy (Gates et al. 1986) and is the major problem threatening the child's quality of life after bilateral tube placement (Debruyne et al. 1988, Rosenfeld et al. 2000). Drainage through the tube within the first few weeks after tympanostomy is called early postoperative otorrhea (Rosenfeld and Bluestone 1999). Several perioperative methods such as prophylactic antibiotic drops after TT placement (Scott and Strunk 1992, Epstein et al. 1992, Salam and Cable 1993, Hester et al. 1995), preoperative external ear canal disinfection (Baldwin and Aland 1990, Giebink et al. 1992), and post-tympanostomy trimethoprim-sulfamethoxatzole and prednisone treatment (Daly et al. 1995) are aimed at preventing early postoperative otorrhea in intubated ears, though degree of surgical asepsis during tube placement has no effect on risk for early postoperative otorrhea (Odutoye et al. 2003). Because one study reported 17 children to be treated with prophylactic ear-drops to prevent only one case of postoperative otorrhea (Rosenfeld and Bluestone 1999) and because of the potential ototoxicity of some ear-drops (Welling et al. 1994), their routine use is not recommended (Garcia et al. 1994). The bacteria found in a tube that is draining are basically the same pathogens that cause the AOM (Mandel et al. 1994). In children older than 3, however, bacteria considered to be the normal flora of the external ear canal occur more often than among the younger patients: Incidence of *Staphylococcus aureus* in the ear discharge can be 39% and of *Pseudomonas aeruginosa* 24% (Schneider 1989). One or both of these bacteria may occur in 44% of otorrhea episodes in a tympanostomized ear (Mandel et al. 1994). In chronic and recurrent tube infections, as well, *Staphylococcus aureus* and *Pseudomonas aeruginosa* occur frequently (Brook et al. 1998).

Early postoperative otorrhea and recurrent otorrhea—with three or more acute episodes—occur more often in ears with MEE present in tympanostomy (Valtonen et al. 1997 and 1999). Chronic otorrhea is associated more with long-term tubes (Slack et al. 1987). Incidence of chronic otorrhea lasting longer than 3 weeks in the tympanostomized ear is 4% to 6% (Rosenfeld and Bluestone 1999). The management of acute, short-term otorrhea is usually simple, and the otorrhea sometimes resolves even without treatment (Herzon 1980). The draining ear is usually cured with saline rinsing and with both per oral and local antibiotic treatment based on bacterial culture (Supance and Bluestone 1983). Studies supporting the effect of per oral prednisolone as an adjuvant therapy in AOM with discharge through the TT also exist (Ruohola et al. 1999). Chronic otorrhea through the tube is often considered a consequence of implant contamination, and this is best cured by tube removal (Luxford and Sheehy 1982, Bingham et al. 1989).

A tympanic membrane irritated by the tube may produce granulation tissue, the most common cause of bloody otorrhea (Rosenfeld and Bluestone 1999), and which occurs in 6% of the ears with titanium tubes (Handler et al. 1988, Cunningham et al. 1993). Atrophy or a sclerotic plaque on the tympanic membrane after tube extrusion are abundant, seen as often as in one-quarter to one-third of all ears treated with

TTs, but these are generally cosmetic problems that rarely cause any morbidity or necessitate procedures (Goldstein et al. 1996, Kay et al. 2001). A chronic perforation of the tympanic membrane occurs in 2.2% of ears treated with conventional short-term tubes; perforations occur more often (12%) in ears treated with the long-term tubes (Weigel et al. 1989, Matt et al. 1991, Kay et al. 2001). The most severe complication of TT treatment, cholesteatoma, occur in 0.7% of the tympanostomized ears (Kay et al. 2001). TT placement itself seems to cause no conductive or sensorineural hearing loss (Emery and Weber 1998). Although many of the TT sequelae such as acute otitis media, otorrhea, tympanosclerosis, focal atrophy, and even cholesteatoma can actually occur in an unoperated ear of an otitis-prone child and thus can rarely be attributed directly to tube treatment, these tend to be more frequent in a tympanostomized ear (Maw and Bawden 1994 b, Kay et al. 2001, Daly et al. 2003).

## 2.3 MEDICAL BIOMATERIALS

### 2.3.1 DEFINITION

Medical biomaterials are the metal, ceramic, and polymer materials or their composites used to build medical devices; a medical implant is any object made of one or more biomaterials that replaces a structure or function of the body (Costerton et al. 1987, DeMane 1995).

The properties of an ideal implant material are, according to DeMane (1995), that it is: noncarcinogenic, non-allergenic, sterilizable, capable of fabrication in the forms desired, capable of forming a living bond to tissue, and of acting as a drug carrier or delivery depot, and capable of controlled release of biologic proteins such as bone morphogenic proteins. Unless the material is biodegradable, it should be clinically inert, structurally stable, not physically modifiable by tissue fluids, and not illiciting any inflammatory or foreign body response.

Today, biomaterial solutions are so usual that in our technologically advanced society almost every human being is host to some biomaterial. Among the most common medical implants, next to dental fillings, are TTs. Biomaterial surfaces may be hydrophobic, like Teflon, plastic, latex, or silicone, or hydrophilic, like glass or metals. These materials are also characterized by their surface texture, roughness or smoothness, and antimicrobial properties. The surface properties that most invite bacterial adherence and biofilm formation are roughness and hydrophobicity. (Donlan 2001).

### 2.3.2 TYMPANOSTOMY TUBES

TTs are constructed of polymers such as silicone, polyethylene, or Teflon, metals such as stainless steel, titanium, or gold, and ceramics such as hydroxylapatite (Heumann et al. 1982, Tami et al. 1987, Shone and Griffith 1990). Hundreds of different tube models exist. Their design varies from short- to long-shafted, with variable sizes and forms for flanges and tube lumens (Tavin et al. 1988, Lindstrom et al. 2004). Functionally, TTs are categorized as short- or long-term tubes by the length of time they are predicted to ventilate the middle ear. Short-term tubes stay open from approximately 8 to 18 months, and long-term tubes more than 15 months (Rosenfeld and Bluestone 1999). The short-term tube is suitable for most patients, with its ventilation time sufficiently long to secure the healing process of the middle ear with an acceptable risk for sequelae. The long-term tube is reserved for the rare cases

needing a long middle ear ventilation period, despite a risk for persistent perforation of the tympanic membrane and a high frequency of otorrhea (Per-Lee 1981, Weigel et al. 1989, Goode 1996,). Long-term T-tubes are indicated, for example, for patients with cleft palate or other craniofacial anomalies leading to a need for lengthy middle ear aeration (Wielinga and Smyth 1990).

Because TT extrusion occurs as keratin from the tympanic membrane accumulates between the tympanic membrane surface and the outer flange of the tube, finally pushing the inner flange out through the tympanic membrane, the main factors leading to late extrusion time are long interflange distance, a large inner flange, and an absent or small outer flange (Gordts et al. 1993, Isaacson and Rosenfeld 1996). A keratin ring around the collar of an extruded TT is often present (Reid et al. 1988). TT occlusion occurs more often if the tube is long-shafted or if the tube lumen diameter is very small, but on the other hand, a larger tube lumen will lead to more tympanic membrane perforations after tube extrusion (Bluestone and Klein 1995, Morris 1999).

The role of the TT is to bypass a poorly functioning eustachian tube, improve hearing, and reduce symptoms caused by AOM such as otalgia. To succeed, the TT lumen must remain patent. The tube itself may become colonized with pathogenic bacteria, resulting in persistent infections, and the tube material may play an important role in development of otorrhea and tube occlusion (Biedlingmaier et al. 1998). The chemical composition and physical characteristics of the surface of an implantable device such as charge, hydrophobicity and roughness are the main factors influencing bacterial adherence and the adhesion of proteins as well as other molecules (An and Friedman 1998). Teflon has low adhesive properties, and Teflon tubes are smoother than silicone tubes in scanning electron microscopy (SEM) pictures; such smoothness reduces bacterial adhesion capacity (Karlan et al. 1980 and 1981, Quirynen et al. 1993). Less otorrhea occurs with Teflon than with silicone tubes (Karlan et al. 1980). Whether this is due to the chemical properties of Teflon or to the smoothness of the tube surface is, however, unknown.

Tube impregnation with a bactericidal component such as silver oxide can reduce the incidence of otorrhea in a tympanostomized ear (Chole and Hubbell 1995, Gourin and Hubbell 1999). Biomaterial surface properties can also be changed with ion implantation that alters the hydrophobicity of a surface and makes it more resistant to adhesion (Sioshansi 1994). Such ionized silicone TTs show at least a short-range resistance to bacterial biofilm formation in guinea pigs compared to that of conventional tubes (Saidi et al. 1999). The smoothness or roughness of the material may also contribute to resistance against bacterial colonization and biofilm formation. Recurrences of otorrhea in an ear with a TT are often connected with typical risk factors for RAOM, but as shown by Chole and Hubbell (1995) the tube material can also contribute to otorrhea.

### 2.3.3 UNDESIRABLE ADHESION WITH BIOMATERIALS

*Bacterial adhesion*

During recent decades, the number of implantable devices has grown (DeMane 1995). The obvious problem with these devices, implant-related infections, has awakened interest in the mechanisms by which the bacterial colonization and subsequent infection develop. As bacteria prefer to grow on a surface rather than suspended in the surrounding medium, bacterial adhesion to the surface and colonization of biomaterial are, in general, the initial steps in development of biofilm formation

and infections on biomaterials (Hogt et al. 1985, Wadstrom 1989).

Adhesion of bacteria to the substratum is divided into two phases. In the first phase, long-range physicochemical interactions such as gravitational forces or forces of surface electrostatic charge, and short-range interactions such as chemical bonds, ionic and dipole interactions, and hydrophobic interactions transport the bacteria close to the surface and facilitate their initial attachment (Fletcher and Loeb 1979, Krekeler et al. 1989, Mafu et al. 1991). In the second phase, stronger adhesion of bacteria to the surface is achieved by the adhesins in the bacterial capsules: fimbriae and slime (An and Friedman 1998). Slime-producing bacterial strains are more adherent and pathogenic than others; they can produce colonies of bacterial biofilm that are strongly anchored to the surface of an implanted device and can resist host defense mechanisms and treatments because of this protective slime cover (Christensen et al. 1982). Moreover, certain bacterial surface components and substrata exhibit specific adherence mechanisms. Such a specific adhesion may also be important in the pathogenesis of prosthetic infections (Timmerman et al. 1991).

Bacterial adhesion is a complex process influenced by a material's surface properties, by factors in the surrounding medium and by environmental factors such as temperature, bacterial concentration, exposure time, and the presence of antibiotics (Ørstavik 1977, Gristina 1987), as well as by characteristics of the bacterial species and strains. Furthermore, a totally implanted device is rapidly coated with surrounding proteins and immunocomplexes that can mask the properties of the underlying surface itself and promote bacterial adhesion (Eberhart et al. 1987, An and Friedman 1998).

Differences in characteristics of hydrophobicity and the surface charge of bacteria vary between species and strains (Hogt et al. 1985). Hydrophobic interactions influence the adherence of hydrophobic bacteria more than they influence hydrophilic ones; in regard to hydrophobic interactions, similar bacteria prefer a similar surface, which in practice means that hydrophobic bacteria adhere more eagerly to a hydrophobic surface (Satou et al. 1988). As to adherence of hydrophilic bacteria, electrostatic interactions seem to be more important (Satou et al. 1988). The bacterial surface charge in an aqueous medium is negative, but this negative charge is higher for hydrophilic bacteria (Hogt et al. 1985).

Surface chemical composition plays a major role in bacterial adhesion to medical devices such as sutures (Chu and Williams 1984). Metal surfaces are hydrophilic; polymers such as polyethylene and Teflon are hydrophobic (An and Friedman 1998). Basically, surface hydrophobicity leads to increased bacterial adhesion, but some bacteria have unique adhesion affinities for certain materials (Timmerman et al. 1991). For example, *Staphylococcus epidermidis* adheres more easily to polymers and *Staphylococcus aureus* more easily to metal surfaces (An and Friedman 1998). The overall surface area and configuration also influence bacterial adhesion. Rough surfaces and braided, porous, or grid-like surface configurations have a greater surface area and provide more depressions for safe colonization; this also influences biofilm formation (Chu and Williams 1984, Quirynen et al. 1993, Verheyen et al. 1993). The surface charge also has some influence on bacterial adherence (Emery et al. 2003) although this is less important than surface hydrophobicity (An and Friedman 1998).

*Biofilm formation*

An accumulated mass of micro-organisms and their extracellular slime material of proteins, polysaccharides, or both on a solid surface is called biofilm (An and Friedman 1998). Biofilm forms on any kind of material when bacteria adhere to surfaces in aqueous environments and begin to excrete a slimy, glue-like substance that can anchor them and provide protection from phagosytosis and other host defense mechanisms (Barth et al. 1989, Costerton and Stewart 2001). The bacteria in biofilm display a different phenotype than do free-living bacteria (Costerton et al. 1995, Erdos et al. 2003), and their metabolic and division rate is greatly reduced, leading to resistance against antibiotics and against standard bacterial cultures (Fitzgerald and Williams 1975). A biofilm can be formed by a single bacterial species, but more often biofilms consist of many species of bacteria, debris, and corrosion products (Costerton et al. 1999). Antibiotic treatment is able to affect the peripheral bacteria in biofilm, but deeper organisms are resistant to virtually all treatment and act as a center for regrowth and periodic bacterial bursts that lead to new bouts of acute infections (Stewart and Costerton 2001, Ehrlich et al. 2002). Furthermore, susceptibility to antimicrobial agents can be diminished by the active exchange of resistance plasmids that occurs in biofilms (Donlan 2001). For these reasons, recurrent implant-derived infections are best cured with removal of the implanted device, but this leads to increases in time and costs for treatment, not to mention discomfort for the patient (Gristina 1987, Donlan 2001). Although it has been known since the 1970s that most of the bacteria in nature live in biofilm colonies, knowledge of infectious diseases is mainly based on studies made with free-floating, planctonic bacteria (Manning 2003).

During recent years, more evidence of the presence of biofilm in AOM and OME has been gathered (Post 2001, Fergie et al. 2004). This biofilm formation occurs in chronically infected in-dwelling medical devices such as voice prostheses (Everaert et al. 1999, Free et al. 2001) and TTs (Biedlingmaier et al. 1998, Post 2001). The resistance of these biofilm bacteria to antibiotic treatment can explain the failure of antibiotic treatment in chronically draining TT and the fact that otorrhea in a tube ear is usually cured by removal of the tube. Furthermore, evidence exists for the presence of bacterial biofilm formation in human cholesteatoma (Chole and Faddis 2002).

*Protein and platelet adhesion and blood clotting*

Protein adhesion on a biomaterial surface is based on physical and chemical interactions similar to those of bacterial adherence (An and Friedman 1997). Protein adhesion is important because when biomaterial is exposed to blood or extracellular fluid, almost immediately a surface layer of proteins form (Chang 1973, Brynda et al. 2000) that can affect the subsequent adsorption of micro-organisms and platelets on the surface (Vaudaux et al. 1984, Herrmann et al. 1988). One of the typical proteins able to adhere to an implant surface and bind bacteria is fibronectin (Mosher and Proctor 1980, Kuusela et al. 1985), a cell-glue protein distributed by all body fluids (Vaheri and Salonen 1988). On the other hand, some adhered proteins such as albumin change bioimplants' surface properties so that adhesion of other molecules and initiation of coagulation response is inhibited (Hyde et al. 1999). In intravenous devices, the biomaterial can be designed to favor the albumin adhesion essential for their hemocompatibility (Mantero et al. 2001).

An especially problematic form of unwanted adhesion on synthetic materials exposed to circulating blood (Chang 1973) is platelet adhesion and the subsequent surface-induced thrombosis. In intravenous implants such as catheters, the prevention of such adhesion is crucial for implant function. Furthermore, platelet adhesion can mediate bacterial adhesion on a surface, and this can finally lead to septic embolisms (Wang et al. 1993).

### 2.3.4 PREVENTION OF UNDESIRABLE ADHESION

Because in practice all implanted biomaterial devices are naturally exposed to body fluids such as blood, saliva, pus, or extracellular fluid and their proteins and other components that can facilitate bacterial adhesion and biofilm formation, the biomaterials are sometimes pretreated with molecules possessing antiadherent or antimicrobial properties (Zdanowski et al. 1993). Surface coatings can influence bacterial adhesion by altering implant-surface properties such as charge or hydrophobicity (Brokke et al. 1991). An antiadherent coating is characterized by its strong adherence to surfaces and its ability to prevent adhesion of cells and other biological substances simultaneously without its having any actual antimicrobial properties (Hyde et al. 1999). The absorbed protein layer reduces surface hydrophobicity and raises net negative charge and thus reduces adhesion of both hydrophobic and hydrophilic bacteria that are both negatively charged (Hogt et al. 1985). This is the theoretical basis for albumin coating. Changes in surface charge and hydrophobicity can be achieved also by ion-coating of the surface (Sioshansi 1994). Biedlingmaier et al. have shown that biofilm formation may be reduced in implants coated with bombarding ions, although they have made no quantitative comparisons between this and other methods (Biedlingmaier et al. 1998, Saidi et al. 1999).

An antimicrobial coating can diminish bacterial adherence on an implant surface. Silver oxide (AgO) antimicrobial coating has been effective in urinary tract catheters and TTs (Lundeberg 1986, Chole and Hubbell 1995).

#### *Albumin coating*

A typical example of antiadherent coating is albumin. Albumin is the main plasma protein and has a high net negative charge and strong surface-binding activity (Tullis 1977a, b). Albumin produces a smooth monolayer coating (Ton et al. 1979) that inhibits bacterial and other adhesion on the surface by increasing the surface negative charge that promotes electrostatic repulsion between bacteria and surface, and by reducing the surface hydrophobicity that diminishes adherence of the more adhesive hydrophobic bacteria (Hogt et al. 1985, Reynolds and Wong 1983, Brokke et al. 1991). Surface coating with albumin also prevents adhesive contact by blocking active binding sites on the surface and by binding and inactivating part of the normally contact-activated plasma proteins (Eberhart et al. 1987). Albumin coating can be achieved by suspending the material preoperatively in an albumin solution (An et al. 1996) or, as in some intravenously implanted devices, by coating the surface with albumin-binding molecules such as carbon alkyl chains that rapidly and selectively bind albumin from the bloodstream (Chang 1969, Eberhart et al. 1987).

Characteristic for albumin is its strong surface adherence by hydrophobic interaction (Tullis 1977a). Inhibition of bacterial adherence on an albumin-coated titanium surface has been shown to remain higher than 85% throughout a 20-day study of tita-

nium plates implanted in rabbits (An et al. 1997). A cross-linking agent, usually carbodi-imide, is sometimes used with the albumin coating in arterial grafts or orthopedic implants (Ben Slimane et al. 1988 a and 1988 b). Chang has shown that albumin, even without a cross-linking agent, attaches firmly to the surfaces of a hemoperfusion system and produces a very thin and smooth layer, not displaced by fibrinogen in the circulating blood, even with a high blood flow-rate of 300 ml/min (Chang 1974).

The effect of a serum albumin coating in preventing platelet aggregation and blood-clot formation on a biomaterial surface was first reported in 1967 by Packham and colleagues and introduced in more detail 2 years later (Chang 1969, Packham et al. 1969). Since these first *in vivo* tests, coating of implants with human serum albumin (HSA) has been investigated actively in several medical fields both to repel unwanted adhesion on artificial implant materials and to prevent bleeding through porous vascular prostheses at implantation (Chang 1974, Remuzzi and Boccardo 1993, al-Khaffaf and Charlesworth 1996, Brynda et al. 2000). The antithrombogenic properties of albumin coating have been tested both in extracorporeal hemoperfusion devices and in artificial heart valves (Chang 1974, Hyde et al. 1999). Albumin coating also prevents fibrinogen and IgG adsorption on artificial surfaces in extracorporeal hemodialyzer membranes and pacemaker leads, and prevents bacterial as well as leukocyte adhesion on titanium implants and vascular prostheses made of polytetrafluoroethylene and Dacron (Lubin et al. 1986, Kottke-Marchant et al. 1989, Zdanowski et al. 1993, McDowell et al. 1995, An et al. 1996). The inhibitory effect of albumin coating on bacterial adherence and of postoperative infections on orthopedic titanium implants has been tested both *in vitro* and *in vivo* (An et al. 1996 and 1997).

Commercially available HSA is normally concentrated from donor blood. Because albumin is an exceptionally stabile protein, it is routinely processed with 60 °C heating for several hours (Tullis 1977 a). As a result of this pasteurization, all known viral pathogens are eradicated (Erstad 1996). Moreover, blood donors in Finland are tested to assure that they are negative for HIV and hepatitis viruses. Albumin has excellent viral safety records despite over 60 years of its abundant intravenous use as a plasma expander (Erstad 1996), which has in recent years amounted to over 5 million doses annually worldwide (Vincent et al. 2003). The rarely occurring hazards connected with use of albumin are based on anaphylactic reactions in high-dose intravenous infusions (Vincent et al. 2003).

When biocompatibility of albumin as an implant coating was tested, no significant difference appeared in total circulating T cells or in the IL-2 receptor-positive cell subset between rats with albumin-coated and uncoated vascular grafts implanted intraperitoneally. This was true even when the grafts were coated with xenogenic human serum albumin, which represents a more severe immunological challenge (Marois et al. 1996). HSA has served as a surface coating in arterial grafts for over a decade. In a 5-year follow-up study of albumin-coated vascular prostheses, no albumin-derived viral infections or other disadvantages occurred in a group of 200 patients (al-Khaffaf and Charlesworth 1996).

# 3
# AIMS OF THE STUDY

Because undesirable adherence of bacteria and proteins of effusion and blood on TT surfaces can cause tube occlusions and recurrent infections that often lead to tube removal, this study was designed to gain experience as to the effect of an antiadherent albumin coating in reducing TT sequelae.

**Specific questions to be answered:**

1. What is the effect of albumin coating in preventing adherence of fibronectin, the most abundant "cell glue" in exudates, on the surface of five standard TTs, each made of different material?

2. Do the antiadherent properties of albumin-coated TTs survive during 8-month storage of the tube in different environments?

3. Can albumin coating prevent bacterial adherence on a titanium surface?

4. What is the effect of albumin coating on tube sequelae in a patient with an albumin-coated TT in one ear and an uncoated, but otherwise identical tube in the other ear in a prospective, double-blind clinical trial?

# 4
# MATERIALS AND METHODS

## 4.1 TYMPANOSTOMY TUBES (I, II, IV)

All TTs used in the experiments were commercially available tubes manufactured by Medtronic Xomed, Inc., in Jacksonville, FL, USA. The tube models, materials, and the Roman numeral of the study in which they were used appear in Table I. Two (I) or three (II) HSA-coated tubes were compared to an equal number of uncoated tubes in the *in vitro* experiments. In the clinical trial (IV) each child received an HSA-coated titanium tube in one ear and an uncoated, otherwise identical titanium tube in the other.

## 4.2 TITANIUM PLATES (III)

Bacterial adhesion on HSA-coated surfaces was tested with titanium plates because the complex shape of TTs makes quantification of the adhered bacteria unreliable. Commercially pure titanium plates of size 5 x 5 x 1.2 mm were polished with a silicone carbide polishing paper up to P 1200 so they all had identical surface structure under light microscopy prior to exposure to bacteria. Three HSA-coated and uncoated plates were used to test the adherence of each bacteria.

## 4.3 HUMAN SERUM ALBUMIN (HSA)

In the first *in vitro* experiment it was evident that an extremely small amount of serum albumin is sufficient to coat fully a TT surface. HSA produced by the Finnish Red Cross Blood Transfusion Service was the choice for the clinical trial because it is tested for medical use. Coating methods with the cross-linking introduced earlier by other authors were also tested. Altogether, the four methods to obtain the albumin coating were:

1. Albumin coating with 0.01% HSA resulted when 200 µl purified HSA (Sigma, St. Louis, MO, USA) in a concentration of 0.1 mg/ml was used to immobilize the albumin on the TTs surfaces at room temperature overnight (I, II).

**Table I.** TTs in Studies I, II, and IV.

| Tube model | Material | Study |
|---|---|---|
| Soileau Tytan | Titanium | I, II, IV |
| Donaldson Type Silicone | Silicone | I, II |
| Donaldson Type Fluoroplastic | Teflon | I |
| Donaldson Type C-flex | Thermoplastic elastomer | I |
| Donaldson Type Activent | Silicone with AgO coating | I |

2. Albumin coating with 1% HSA resulted when 250 µl purified HSA (Human Albumin SPR 40 mg/ml, Finnish Red Cross Blood Transfusion Service, Helsinki, Finland) at a concentration of 40 mg/ml, diluted with 750 µl phosphate-buffered saline (PBS), was used to immobilize the albumin on a titanium plate (III) or titanium tube (IV) surface at room temperature overnight.

3. Albumin coating with 2% HSA, supplemented with cross-linked carbodiimide, resulted from soaking silicone tubes in a mixture of 1.0 ml purified 4% HSA (Sigma) and 1.0 ml of 0.2% M carbodi-imide (Sigma) for 10 minutes to immobilize the albumin with its cross-linkage on the TT surface (An et al. 1996), (II).

4. Albumin coating with 4% HSA resulted when 200 µl purified HSA (Human Albumin SPR 40 mg/ml, Finnish Red Cross Blood Transfusion Service) at a concentration of 40 mg/ml was used to immobilize the albumin on the TT surface at room temperature overnight (II).

### 4.4 FIBRONECTIN (I, II)

Fibronectin, a typical protein in serum and exudates known as one of the most adherent glycoproteins, is also called "cell glue." It is widely distributed in all body fluids as well as in connective tissue matrices in soluble and insoluble form (Vaheri and Salonen 1988). Fibronectin served as a model to represent glue proteins in exudates of the ear in studies I and II. Fibronectin, 200 µg suspended in 200 µl of Tris-buffer containing 1 mM EDTA, was radiolabeled with 0.5 mCi of $^{125}$I in Iodogen-tubes according to the Iodo-Gen method (Pierce Chemical Co., Milwaukee, WI, USA). The free iodine was removed from the radiolabeled fibronectin on a G-25 gel chromatography column (PD-10, Pharmacia, Täby, Sweden). To detect fibronectin adhesion on the TT surfaces, a duplicate (I) or triplicate (II) of five different tube types (Table I), either coated with HSA or uncoated, was incubated with 800 ng of $^{125}$I-labeled fibronectin overnight. The bound radioactivity was counted after several washes with a gamma counter (Wallac, Turku, Finland).

### 4.5 TUBE STORAGE (II)

To test the durability of the antiadherent properties of the HSA coating on the TT surface, silicone tubes (Medtronic Xomed) were stored for 2 weeks, 4 weeks, 3 months, and 8 months. To mimic the conditions that TTs and tube coatings must normally endure, the trial was carried out in three different environments: a dry +4 °C, a dry +37 °C, and a humid +37 °C. After storage, binding of radiolabeled fibronectin on HSA-coated and uncoated tubes was tested. Because in warm, humid conditions bacterial contamination of test devices happens almost inevitably if the test is continued for months, the study was halted at 4 weeks in the humid +37 °C environment. The experiment was organized so that the fibronectin adhesion test was made for all the tube groups at the same time. The tubes stored for 8 months were put first in the storage chambers 8 months before the actual adhesion test, and the tubes to be stored for one month were put into storage last, 7 months later, one month before the adhesion test.

## 4.6 SCANNING ELECTRON MICROSCOPY (SEM)

A scanning electron microscope Zeiss DSM 962 (Oberkochen, Germany) was used to visualize TT surface changes after coating of the tubes with HSA. Titanium and silicone tubes (Medtronic Xomed) tested were prepared for the SEM with a platinum coating. Magnifications were x50 and x500 (II).

A pair of TTs that extruded from each ear of one patient after 9 months in the clinical trial (IV), after preparation with gold coating, were viewed with JEOL JSM 6335F (JEOL USA, Inc. Peabody, MA, USA) SEM. The magnifications were x100 and x500.

## 4.7 BACTERIA (III)

Adherence of typical pathogens causing severe contamination problems in TTs was tested in an *in vitro* experiment. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were chosen to represent these bacteria because they are able to form biofilm and are over-represented in chronic and recurrent infections of ears with TTs (Brook et al. 1998, Mandel et al. 1994). The bacteria were commercial strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *S. aureus* (ATCC 25923) was cultured in Todd-Hewitt broth at +37 °C overnight under continuous orbital shaking; *P. aeruginosa* (ATCC 27853) was cultured at +37 °C overnight on chocolate plates made in Myller-Hinton II broth (19 g/l) and trypticase soy agar (29 g/l) (Becton, Dickinson & Co. Cockeysville, MD, USA). Both bacteria were suspended in PBS to give the final concentration of 1—5 x $10^8$ colony-forming units /ml (CFU/ml), based on optical density at 600 nm (*S. aureus*) or as confirmed by plate counts (*P. aeruginosa*).

Three HSA-coated and three uncoated titanium plates were exposed to the bacteria in a suspension at room temperature for 90 minutes. Plates were rinsed with PBS and aqua, dried at room temperature, and thereafter dyed with acridine orange prior to quantification of the bacteria under fluorescence microscopy.

## 4.8 FLUORECENCE MICROSCOPY AND QUANTIFICATION OF BACTERIA (III)

The titanium plates, viewed with the fluorescence microscope Zeiss Axioplan 2 (Carl Zeiss Vision GmbH, München-Hallbergmoos, Germany) at x63 and 10 fields sized 428 x 339 µm (1300 x 1030 pixels), were photographed with a Zeiss AxioCam HRc (Carl Zeiss). The picture-processing program ImageJ (National Institute of Health, Washington, DC, USA) was used to calculate the amount of surface area coated with fluorescent bacteria.

## 4.9 PATIENTS AND PROSPECTIVE STUDY DESIGN (IV)

Altogether 179 children undergoing bilateral tympanostomy due to bilateral OME or RAOM were enrolled in the prospective, double-blind clinical study with a 9-month follow-up. Inclusion criteria were: MEE without symptoms of AOM persisting at least 3 months (OME) or three or more AOM attacks during the preceding 6 months (RAOM). Patients were excluded from the trial if they had unilateral otologic problems. Into each child in the study was inserted one uncoated titanium tube and one HSA-coated but otherwise identical tube. These were randomized so that the HSA-coated tube was inserted into either the left or right ear. Because HSA coating is visible only under high magnification in SEM (II), the operation and follow-up were truly double blind.

Of the 179 patients, 146 were operated on at the Department of Otorhinolaryngology at Helsinki University Central Hospital (HUCH), and the other 33 were operated on at Jorvi Hospital in Espoo. Nine patients (5%) were lost to follow-up, so statistical analysis was based on data from 170 patients; of these, 108 (64%) were male and 62 (36%) female. Ages ranged from 6 months to 15 years, median age 3.8 years. The number of primary tube insertions was 106 (62%) and that of secondary tube insertions 64 (38%). All patients had satisfied the inclusion criteria: RAOM for 63 (37%), and OME for 107 (63%) patients.

In the surgical procedure, the ear canal was cleaned, an anteroinferior quadrant incision was made in the tympanic membrane, and any middle ear effusion was aspirated before the tube was inserted through the tympanic membrane. The patency of the TT was secured with suction if needed. Any excessive bleeding requiring suction or treatment with adrenalin was recorded. In the same operation, adenoidectomy was performed on 94 (55%) of the patients.

In postoperative follow-up, the patients were examined by an ear, nose, and throat (ENT) specialist at 1, 3, 6, and 9 months after the procedure. Follow-up of the 146 patients undergoing surgery at HUCH was done by the author at HUCH. The patients having surgery at Jorvi Hospital were followed up by the author and other ENT specialists at the Leppävaara Health Care Center in Espoo. At each visit, number of AOM attacks and drainage time of the possible otorrhea in each ear, results of bacterial cultures, and number of upper respiratory infections since the previous examination were documented. Pneumatic otoscopy and tympanometric evaluation with the GSI 38 Auto Tymp (Grason-Stadler, Inc. Milford, NH, USA) were performed to evaluate TT and middle ear status. In selected cases, otomicroscopy or audiometry was done if needed. Ears with otorrhea were treated with suction and saline irrigation repeated every other day if needed, bacterial cultures were made, and the patients were treated with conventional oral and antibiotic therapy and eardrops. Acute otologic problems between the follow-up examinations were treated at health care centers or private outpatient clinics, according to the Finnish recommendations of good clinical practice.

A tympanometric test was used to evaluate both the middle ear ventilation through the TT and middle ear status after the tube had extruded or occluded. The GSI 38 Auto Tymp tympanometer has an ear canal volume (ECV) function that can assess a possible open air route into the middle ear cavity through the tympanic membrane. ECV is over 1 $cm^3$ in cases with tympanic membrane perforation or open TT because both ear canal volume and volume of the middle ear and mastoid cavity are counted (Shanks et al. 1992). In this respect, in tympanostomized ears three different types of results in tympanometry can appear:

1. Type B (flat) curve and ECV over 1 $cm^3$ as a result of open TT or perforation in the tympanic membrane, allowing air passage to the middle ear.
2. Type A or C curve: Healthy middle ear with occluded or extruded tube.
3. Type B curve and ECV under 1 $cm^3$: OME in ear with occluded or extruded tube.

If the tube inner flange is seen above the tympanic membrane, and tympanometry shows results of type 2 or 3, tube extrusion is diagnosed. Even in cases where the tube is tilted, giving no straight view into the tube lumen, one can distinguish whether the tube is open, blocked, or extruded with these specifications.

## 4.10 ETHICAL ASPECTS

In the prospective study (IV), parents of all the patients enrolled signed an informed consent prior to each study procedure. The study protocol was approved by the Ethics Committee of Helsinki University Central Hospital.

## 4.11 STATISTICAL METHODS

The statistical analyses were performed with the SPSS 9.0 (SPSS, Inc., Chicago, IL, USA) program for all the *in vitro* studies and in analyzing results of the *in vivo* study. The criterion for statistical significance was $\alpha$=0.05 for all experiments.

In the first *in vitro* experiment (I), binding of $^{125}$I -labeled fibronectin was detected on two uncoated and HSA-coated TTs of each tube type. Mean values of the bound fibronectin were compared to find the possible binding inhibition achieved by HSA coating. In the study on the durability of HSA coating (II), the mean values of fibronectin adherence were tested in triplicate, but otherwise identically to the first *in vitro* experiment. Furthermore, in that study, a combined calculation was performed on fibronectin adhesion in all storage environments, comparing groups by the Kruskall-Wallis test; data were presented with standard error of mean.

Study of bacterial adherence (III) was also done in triplicate. Mean values of plate surface areas covered with adhered bacteria were compared.

In the prospective study (IV), prolonged tube ventilation time, as a result of diminished number of early tube occlusions, was chosen as the principal endpoint. The minimum sample size was calculated with the NCSS 2000 statistical program (NCSS Statistical Software, Kaysville, UT, USA). For this calculation, incidence of tube occlusions was determined to be 7% for conventional tubes (Kay et al. 2001) and 1% for HSA-coated tubes as predicted by the results of the first *in vitro* experiment (I). The minimum sample size needed was 170 when the criterion for statistical significance was $\alpha$=0.05 and desired statistical power 1-$\beta$=0.9. For statistical analysis of the results, it was assumed that tube sequelae such as tube occlusion or extrusion occurred midway between the two examination dates, since the patients were examined at intervals from 1 to 3 months. For the dichotomous outcome variables such as tube occlusions, the standard $\chi^2$ test was used. Continuous variables were statistically evaluated with the student *t*-test.

# 5
# RESULTS AND COMMENTS

## 5.1 *IN VITRO* EXPERIMENTS

### 5.1.1 PROTEIN ADHESION STUDIES (I, II)

In the first two experiments, the binding-inhibition test was used to study the anti-adherent properties of HSA coating. Binding inhibition is the reduction in binding of any model protein or other molecule, in this case fibronectin, on a surface, a reduction achieved by the surface treatment.

Experiment I showed that the amount of HSA needed to coat a TT surface fully is extremely small, 1 µg or even less per tube. It also showed that the amount of fibronectin actually needed to saturate the surface of one TT, coated or uncoated, was much less than the 800 ng routinely used in these experiments, and only 2% to 12% of the fibronectin was attached to the tube surfaces.

For all tube materials independent of storage time or environment, HSA coating reduced the binding of fibronectin. Variation per tube in fibronectin binding between different tube models was small. In uncoated tubes, binding ranged from 101 ng (12.6% of the total 800 ng of fibronectin available) for the C-flex tubes to 73 ng (9.1%) for the titanium tube. A larger difference, from 4.5 ng to 101 ng, however, was evident when HSA-coated and uncoated tubes where compared (I, II). When the tubes were not stored, the binding inhibition achieved with HSA coating ranged from 59% to 85%, depending on tube material, (Table II). The highest binding inhibition occurred in titanium and silicone-AgO tubes: 84.6% and 84.2%.

Durability of binding inhibition was tested 1, 3, and 8 months in three different environments that resemble by humidity and temperature the conditions in which the tubes are stored and used. An inhibitory effect of HSA coating on fibronectin adhesion was evident in all environments even after an 8-month storage, although some drop in binding inhibition occurred in warm and humid conditions (Fig. 1). Whether this drop was due to decay of HSA on the surface remains unclear.

**Table II.** Binding inhibition of fibronectin achieved with human serum albumin (HSA) -coating in tympanostomy tubes.

| | |
|---|---|
| C-flex | 69.0% |
| Fluoroplastic | 59.2% |
| Silicone | 68.8% |
| Silicone-AgO | 84.2% |
| Titanium | 84.6% |

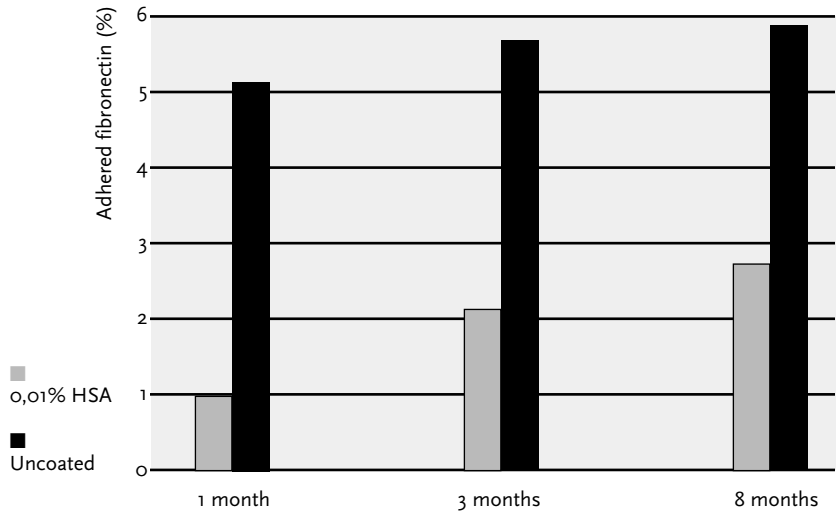


**Fig 1.** Combined results of fibronectin binding on tympanostomy tubes stored under dry conditions at +4°C and +37°C.

In a dry, cold environment, the drop in binding inhibition was small. After an 8-month storage at a dry +4 °C, binding inhibition of fibronectin was 74%. The decline in binding inhibition was more marked when the tubes were stored at a higher temperature. Binding inhibition was 41% after a 3-month storage and 27% after an 8-month storage at a dry +37 °C. At a humid +37 °C, binding inhibition after 4 weeks was 63%. The overall binding of fibronectin on HSA-coated tympanostomy tubes was significantly smaller in all storage environment groups

Of the four HSA coating methods, when the durability of albumin coating with 0.01% HSA and 4% HSA was compared with that of albumin coating with 2% HSA, supplemented with cross-linked carbodiimide, fibronectin binding was higher in the latter, which thus had no advantage over coatings of pure albumin, even in the 8-month trial.

These results show that any TT surface when coated with HSA achieves a strong inhibition of fibronectin binding. The difference in fibronectin adhesion between tube models was in part due to the difference in their total surface area and to their original tube surface characteristics, especially roughness. For example, the difference in fibronectin binding to two uncoated tubes identical in model: silicone (75ng) and silicone-AgO (96ng), can be explained by the fact that the AgO coating increases roughness and thus the surface area of the silicone tube increases.

### 5.1.2 SCANNING ELECTRON MICROSCOPY

In SEM, both silicone and titanium tubes showed some larger grooves, probably due to molding. At small magnification, only a slight difference was evident between coat-

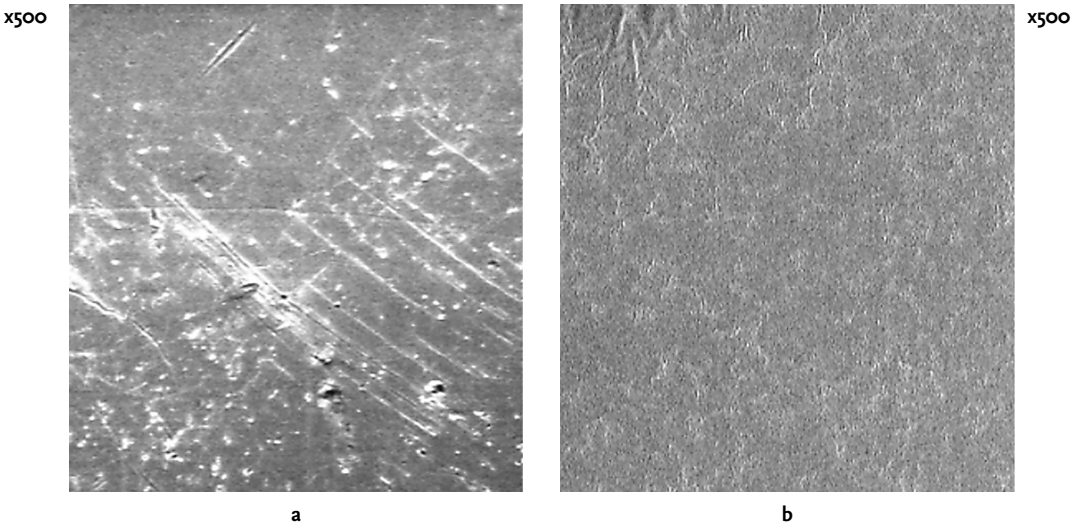


**Fig. 2.** Uncoated (a) and HSA-coated (b) titanium tube surfaces (magnification x500).

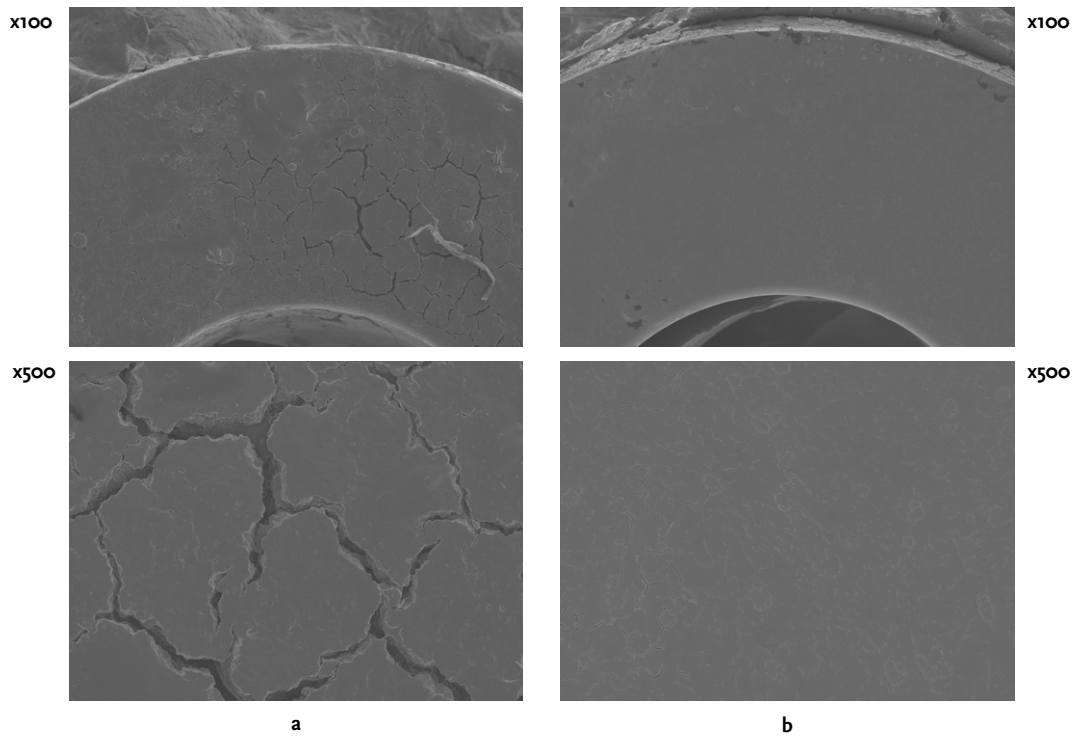


**Fig. 3.** Extruded titanium tubes.
a) Uncoated, b) HSA-coated tubes (magnification x100 and x500).

ed and uncoated tubes. At higher magnifications, differences between the albumin-coated and uncoated tubes could be seen more clearly (Fig. 2). The uncoated surface appeared generally rougher, and some deeper pores and scars could be seen in detail. Albumin coating produces a smooth film that covers the uneven surface of the tubes. The tubes could be seen as densely coated with albumin. Roughness had enlarged the overall surface area of the uncoated tube and thus provided more area for adhesion of foreign materials.

In SEM pictures of extruded titanium tubes, a thick crust on the surface of the uncoated tube and a smooth film-like albumin coating without crust formation on the HSA-coated tube are visible (Fig. 3).

The tubes ventilated normally for 9 months and thereafter were extruded and removed from the ear canal. Preoperatively, this patient had bilateral OME and at surgery mucoid effusion in the ear into which the HSA-coated tube was inserted; the other ear with the uncoated tube had been dry. During follow-up, the patient had no ear infections, and both tubes functioned normally (unpublished data).

SEM pictures of the extruded tubes show that crust formation was inhibited on the albumin-coated titanium surface (unpublished data).

### 5.1.3 BACTERIAL ADHERENCE STUDY (III)

Calculation of bacterial adherence to titanium surfaces was by the picture processing program ImageJ (NIH, USA).

For *Staphylococcus aureus*, 0% to 14.6% of the photographed titanium surface was covered with bacteria (Fig. 4). HSA coating inhibited the adherence of *S. aureus* to the titanium surface significantly in both the $10^8$ and the $5 \times 10^8$ CFU/ml bacterial concentrations tested. For the lower ($10^8$ CFU/ml) bacterial concentration, on average 1.6% of the uncoated and 0.22% of the HSA-coated titanium surface was covered with bacteria, which meant an 82% adherence inhibition. For the higher ($5 \times 10^8$ CFU/ml) bacterial concentration, on average 6.5% of the uncoated and 0.25% of the HSA-coated titanium surface was covered with bacteria, so the adherence was inhib-

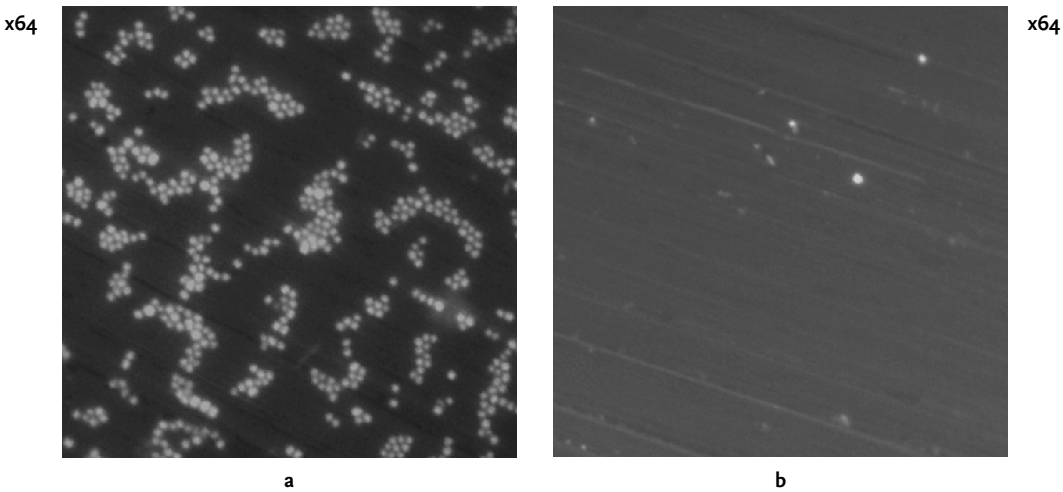


**Fig 4.** Uncoated (a) and HSA-coated (b) titanium plates exposed to *Staphylococcus aureus* dyed with acridine orange (magnification x64).

ited 95%. In each case, the difference was significant ($P < 0.05$).

With *Pseudomonas aeruginosa*, 0.3% to 58.8% of the photographed titanium surface was covered with bacteria (Fig.5). HSA coating reduced adherence of *P. aeruginosa* to the titanium surface in both ($1 \times 10^8$ and $5 \times 10^8$ CFU/ml) bacterial concentrations tested. At the lower concentration, on average 5.0% of the uncoated and 3.3% of the HSA-coated titanium was covered with bacteria, which meant a 29% decrease in adherence achieved with HSA coating. In the higher concentration, in which on average 25.4% of the uncoated and 16.8% of the HSA–coated surface was covered with bacteria, adherence was inhibited 37%. For the higher concentration, this difference was significant ($P < 0.05$). For the lower, $10^8$ CFU/ml concentration, the difference was not significant ($P$=0.09).

Excellent visualization of both bacteria was achieved with acridine orange fluorescent staining. The plate surface area covered with fluorescent bacteria was counted with a picture-processing program.

This study showed that adherence of both *S. aureus* and *P. aeruginosa* to a titanium surface can be inhibited significantly by HSA coating. This inhibition was more marked with *S. aureus* but was also significant at the higher concentration tested with *P. aeruginosa*. The difference in binding inhibition between bacterial species, as also demonstrated here, is possibly due to the fact that the adherence of a bacterium on a surface is dependent not only on the characteristics of the substratum, such as charge and hydrophobicity, but also on the characteristics of the bacterial surface, which vary among bacterial species and strains (An and Friedman 1998).

## 5.2 PROSPECTIVE CLINICAL STUDY (IV)

### 5.2.1 PERIOPERATIVE FINDINGS

Physical appearance of the middle ear contents in this procedure, in the ears with HSA-coated and uncoated tubes, was found to be similar. The surgeon reported excessive perioperative bleeding in 24 (14%) patients, in 15 ears with HSA-coated and

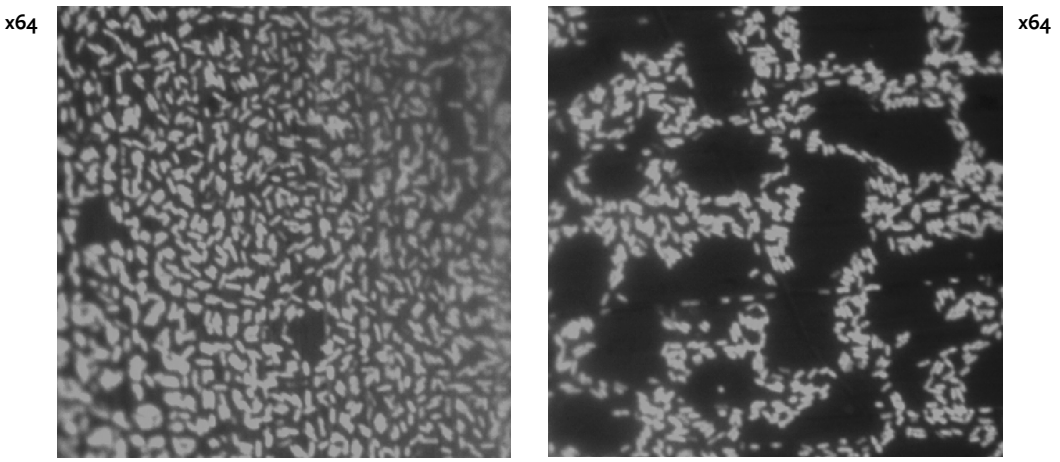


**Fig 5.** Uncoated (a) and HSA-coated (b) titanium plates exposed to *Pseudomonas aeruginosa* dyed with acridine orange (magnification x 64).

in 14 ears with uncoated tubes. Bleeding was always reported when suction or other procedures were needed to stop it.

### 5.2.2 POSTOPERATIVE FINDINGS

Overall ventilation time was longer with HSA-coated tubes (7.1 months) than with uncoated tubes (6.8 months), but with no significant difference. In the 24 patients with excessive bleeding during surgery, a significant prolongation in ventilation time (paired sample *t*-test, $P < 0.05$) occurred in HSA-coated tubes (7.6 months versus 5.8 months).

Otorrhea occurred in 68 (40%) of the patients and in 95 (28%) of the tubes during the 9-month follow-up. No significant difference between HSA-coated and uncoated tubes appeared, although the number of HSA-coated tubes involving otorrhea: 42 (25%), versus 53 (31%) ears, was smaller. An equal numbers of ears, 5 (3%), with recurrent acute otorrhea, meaning 3 or more episodes during the follow-up, had HSA-coated or uncoated tubes. The numbers of chronic otorrhea cases, meaning drainage for more than 3 weeks, were also almost equal in number among HSA-coated and uncoated tubes, 6 (3.5%) and 5 (3%).

Tube occlusion was found at the 3-month follow-up visit significantly less often in HSA-coated tubes than in uncoated tubes, 12 (7%) and 24 (14%) ($\chi^2$ test, $P < 0.05$). At the 9-month follow-up visit, this difference, although again present, was not statistically significant: 59 (34%) to 70 (41%). Most (76%) of the tubes collected at least some dry crust or other impurity during follow-up. In this respect, no difference appeared between coated and uncoated tubes.

Tube extrusion occurred at the 3-month follow-up visit in 7 (4%) of ears with both HSA-coated and uncoated tubes. At the 9-month follow-up visit, 45 (26%) HSA-coated and 39 (23%) uncoated tubes had extruded.

After tube extrusion, tympanosclerotic plaque appeared in a total of 23 (7%) ears, in an almost equal number of ears for extruded HSA-coated and uncoated tubes, 11 (6%) and 12 (7%) ears. Granulation tis-

**Table III.** Sequelae of TT treatment.

| | Ear with HSA-coated tube | Ear with uncoated tube | |
|---|---|---|---|
| Total ears | 170 | 170 | |
| Average tube ventilation time (months) | 7.1 | 6.8 | |
| At least one otorrhea episode | 42 | 53 | |
| Recurrent otorrhea | 5 | 5 | |
| Chronic otorrhea | 5 | 6 | |
| Tube occlusion within 3 months | 12 | 24 | $\chi^2$ test $P < 0.05$ |
| Tube occlusion within 9 months | 59 | 70 | |
| Early tube extrusion within 3 months | 7 | 7 | |
| Tympanosclerosis | 11 | 12 | |
| Granulation | 13 | 17 | |
| Chronic perforation | 0 | 1 | |

sue appeared around the tube in a total of 30 (9%) of the ears, in 13 (8%) ears with an HSA-coated tube and 17 (10%) with an uncoated tube (n.s.). Chronic perforation of the tympanic membrane occurred in one (0.3%) ear that had had an uncoated tube. No cholesteatoma occurred in these patients. Sequelae found in the trial are listed in Table III.

As predicted by the results of the *in vitro* experiments, a reduction in number of tubes with occlusion and in cases of otorrhea occurred with use of HSA coating. Tube occlusion was diagnosed when a tube had crust in its lumen, the inner tube flange was behind the tympanic membrane, and ECV was under 1 $cm^3$ in tympanometry. The results show that the number of occlusions in HSA-coated tubes at the 3-month follow-up visit was half the number of occlusions in uncoated tubes. Furthermore, among the 24 patients with perioperative bleeding, ventilation time with the HSA-coated tube was 31% longer. The number of occluded TTs seemed to rise during follow-up. After one month it was 17 (5%), at the 3-month visit 36 (11%), at the 6-month visit 75 (22%), and finally at the 9-month visit 129 (38%). Because the healing process of the tympanic membrane in some cases seems to repair the tympanic membrane perforation under the tube while the tube is still in the tympanic membrane—leading to ECV under 1 $cm^3$ in tympanometry, and thus satisfying the diagnostic criteria for an occluded tube—the number of tube occlusions may have been overestimated after the first 6 months of follow-up. The difference in tube occlusion between the coated and uncoated tubes was more obvious in the first months of follow-up, and thereafter the difference became smaller, which may be due to gradual decay of the coating or to the fact that eventually almost all tubes are covered with crust which may make their HSA coating ineffective.

# 6
# GENERAL DISCUSSION

Acute otitis media is an extremely common disease among children, with many children suffering from recurrent attacks. Otitis media with effusion is a typical outcome of AOM and one of the most common reasons for hearing loss among children. A great amount of energy is focused on the diagnosis, treatment, and follow-up of pediatric ear diseases all of which place an enormous financial burden on families and the community. Tympanostomy has become the treatment of choice in any OME unresponsive to nonsurgical treatment that has lasted for 3 months, and it is widely used also in treating recurrent AOM. In recent decades, tympanostomy has become one of the most common surgical operations under general anesthesia among children in many countries, and several million TTs are manufactured yearly worldwide. Recently, bacterial antibiotic resistance has made the role of surgical procedures in treating RAOM and OME even more important (Bluestone 1998).

All otologists are familiar with the frustrating problems involving functioning of TTs: occlusion of the tube and otorrhea. This thesis directed its focus toward the most critical reasons for sequelae in the tympanostomized ear: adhesion of cell glue protein and bacteria on the TT surface. One surface mechanical solution for this problem, albumin coating, was tested. The project was originally initiated to create a coating method to achieve antiadherent properties in otological devices. The first *in vitro* experiment on an HSA coating found it to cause excellent inhibition of fibronectin binding onto TTs (I). This experiment showed the potential benefits of the method and was preliminary to *in vitro* and *in vivo* experiments. The principal endpoint chosen for the whole project was prevention of unwanted adhesion on the tube surface, leading to prolonged tube ventilation time. Investigations of TTs emphasizing tube surface characteristics exist to only a very limited extent. In this respect, two surface treatment methods were introduced earlier on: Silver-oxide coating and surface treatment with bombarded ions. Chole and Hubbell (1995) showed that otorrhea occurs less often in TTs coated with AgO. The Biedlingmaier group showed that an ion-treated TT surface can repel biofilm formation (Biedlingmaier et al. 1998, Saidi et al. 1999), but the incidence of TT occlusion in these experiments remains unreported.

HSA possesses excellent antiadherent properties that are useful in hemodialysis devices to reduce platelet adherence on artificial surfaces. HSA-precoated arterial grafts in clinical use can prevent blood leakage through the porous graft immediately after an operation. They have also shown promising results in reduction of bacterial and other undesirable adhesion on arterial grafts and orthopedic titanium implants. Although HSA comes from donor blood, it is safe from blood-derived viral pathogens because, unlike the other blood products, it can be pasteurized at +60 °C for over 10 hours (Tullis 1977a). HSA has an excellent safety record despite its abundant use as a plasma expander for the last 60 years. In recent years, worldwide annual intravenous use of HSA has been over 5 million doses. Furthermore, the biocompatibility of HSA coating on arterial grafts has been tested in a 5-year follow up study (Marois et al. 1996).

## 6.1 *IN VITRO* EXPERIMENTS (I, II, III)

### 6.1.1 STUDIES ON FIBRONECTIN ADHESION

It is known that HSA coating reduces adherence of proteins such as fibrinogen on biomaterial surfaces (Kottke-Marchant et al. 1989), and fibronectin was chosen for testing because it excellently represents the adhesion properties of body fluids (Vaheri and Salonen 1988). Moreover, studies prior to the first *in vitro* experiment showed that the crust adhering to the TT surface consists mostly of proteins such as fibronectin (unpublished data). The fibronectin binding test adopted from earlier studies (Cannas et al. 1990) allowed testing of this new treatment to prevent protein adhesion and crust formation on TT surfaces. Fibronectin adherence diminished significantly (59%-85%) with HSA coating in all tube materials tested, even compared with Teflon, well known for its antiadherent properties also in TTs (Karlan et al. 1980). The number of tube types tested was limited to five. Although samples per group numbered only two, which could lead to bias, the difference was significant for all tube types. That gold tubes were unavailable for testing is frustrating, especially when in Finland use of gold or gold-plated TTs has recently increased.

Because to be useful the favorable properties of any surface treatment should survive long-term storage and wear in the ear in many environments, a durability trial tested binding inhibition of fibronectin by HSA after storage for 1, 3, and 8 months. Although some increase occurred in all tubes in fibronectin binding during this storage time, endurance of binding inhibition was high when the tubes were stored at +4°C. Some decline in binding inhibition occurred at +37 °C. Because TTs have little contact with surrounding tissue, less wear of the surface coating is expected than in totally implanted biomaterials such as orthopedic implants, although some HSA coating may gradually decay off the tube surface.

Three coating methods comprised the one from the first *in vitro* experiment, a 0.01% HSA coating, versus an HSA coating (2%) with a carbodi-imide cross-link introduced earlier by An et al. (1996) to show whether cross-linking enhances durability (Ben Slimane et al. 1988a), versus the new coating method with HSA manufactured by the Finnish Red Cross Blood Transfusion Service to be used in the clinical trial. No major difference appeared between the three methods. The HSA coating with carbodi-imide cross-linking showed no advantage over the use of pure albumin coating in an 8-month trial; cross-linking of albumin coating has not been previously compared to coatings without cross-linking. An and colleagues (1996) found similar durability in a shorter trial of bovine serum albumin-coated titanium cylinders kept for 20 days at +37 °C in PBS with intermittent agitation: Inhibition of albumin coating on *Staphylococcus epidermidis* adherence remained greater than 85%. Durability of other surface treatments on TTs has not been reported. Because the present experiment included only silicone TTs, these results are not comparable to those with other materials. The number of tubes in each study group was raised here to three, to avoid any bias due to small sample number.

### 6.1.2 SCANNING ELECTRON MICROSCOPY

Differences between uncoated and HSA-coated tubes in SEM pictures were obvious (Fig. 2). In the extruded tube (Fig. 3), is visible a thick layer of dry crust on the uncoated tube surface, although this patient did not suffer from otorrhea during follow-up

and had perioperative effusion only in the ear with the HSA-coated tube. No further conclusions can be drawn from pictures of only one pair of TTs, although they support the theory of Karlan et al. (1980), who has shown in SEM pictures that the surface character of a TT has an influence on bacterial adhesion and that subsequent otorrhea occurs less often in ears with smoother Teflon tubes. Earlier, similar findings on the character of crust formation in extruded TTs are from Reid et al (1988) with light microscopy and Saidi et al. (1999) with SEM.

### 6.1.3 STUDIES ON BACTERIAL ADHERENCE

Few if any papers quantitatively compare bacterial adherence on different TT surfaces, although some studies describe biofilm formation and bacterial adherence on tube surfaces (Karlan et al. 1980, Biedlingmaier et al. 1998). The reason for so few studies may be the same as in this thesis: A double-flanged grommet TT is extremely complex in form for reliable detection of number of bacteria adhered to its surface. For testing bacterial adherence on an HSA-coated titanium surface, titanium plates instead of the tubes were thus chosen. Earlier tests of *S. aureus*, and *S. epidermidis* adhesion on bovine serum albumin-coated orthopedic titanium implants *in vitro* found that adherence can be inhibited by 90% with albumin coating (McDowell et al. 1995). Furthermore, to test the *in vivo* effect of albumin coating in orthopedic titanium implants (An et al. 1997), titanium implants, after being incubated in *S. epidermidis*, were inserted subcutaneously into rabbits. Postoperative wound infection occurred in 62% of the rabbits receiving uncoated titanium implants and in only 27% with bovine serum albumin-coated titanium implants.

In the present *in vitro* experiment on bacterial adherence, the two most common bacteria in chronic infections of tympanostomized ears were used, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Adherence of *S. aureus* was inhibited from 82% to 95% depending on concentration of the bacterial suspension. Adherence of *P. aeruginosa* was inhibited from 29% to 37%. The results with *S. aureus* were identical with results of McDowell et al. (1995). The difference in adherence on an HSA-coated surface between these two bacterial species can be due to the fact that their adherence is dependent on both substratum, and bacterial surface characteristics (An and Friedman 1998).

An *in vitro* experiment like this has its limitations. Exposure of one bacterial species to an implant surface without biofilm formation would be a very unusual situation *in vivo*. The *in vivo* lifecycle of an implanted device is a different and far more complex process than the situation *in vitro;* the former includes the simultaneous influence of several bacteria, as well as biochemical and mechanical stress, not to mention the effects of the host defense mechanism. These in vitro results, however, strongly indicate that biofilm formation can also be reduced on pretreated titanium surfaces.

## 6.2 *IN VIVO* STUDY (IV)

The principle of comparing two TT in the contralateral ears of each patient is widely used (Handler et al. 1986 and 1988, Chole and Hubbell 1995). In half the clinical, controlled TT studies, the tubes are compared between contralateral ears and in the other half compared in a parallel group of patients (Kay et al. 2001). In the present prospective trial, inclusion criteria were based on recommendations (Bluestone and

Klein 1995) of practitioning otologists and also from many clinical trials (Gordts et al. 1993).

Although albumin coating of implantable materials has been investigated for several decades, few if any prospective, randomized clinical trials have appeared. A 5-year follow-up of 200 patients with HSA-coated arterial grafts has shown good results, although with no comparison of other methods included (Al-Khaffaf and Charlesworth 1996). Several retrospective and prospective trials compare two or more TTs. Because an obvious difference usually exists between the TTs compared, all these trials except one (Chole and Hubbell 1995), were not double-blind. Another basic problem in these past experiments is the fact that conclusions focus mostly on one feature of the tube, usually its material, such as gold or titanium (Handler et al. 1986 and 1988), although the tubes compared differ in several features such as lumen size, tube form, or length. Sometimes other features than the one focused upon are not even reported (Gordts et al. 1993). This fact may have led to unreliable assumptions stemming from such reports.

In the present prospective study (IV), perioperative findings were similar to those of many follow-up trials (Chole and Hubbel 1995, Cunningham and Harley 1991), although differences may exist between inclusion criteria—difference seldom presented in the older reports. Furthermore, the present findings are in accordance with findings of the clinical trials excellently combined in a meta-analysis based on 134 articles (Kay et al. 2001) (Table IV).

The principal endpoint of the clinical study was prolonged ventilation time resulting from a lower number of tube occlusions; occlusions, according to Kay et al. (2001), are the most frequent adverse side-

**Table IV.** Sequelae of tympanostomy tube treatment.

| | **Kinnari et al. (2004) 340 ears** | **Kay et al. meta-analysis (2001) 13 760 ears** | |
|---|---|---|---|
| **Sequelae** | **Incidence** | **Incidence** | **Range** |
| | % | % | % |
| At least one otorrhea episode | 28 | 17 | 3.0–74.0 |
| Recurrent otorrhea | 3.0 | 2.1 | 1.2–3.4 |
| Chronic otorrhea | 3.2 | 3.3 | 1.9–7.7 |
| Tube occlusion | 37 | 6.9 | 0–37.3 |
| Early tube extrusion within 3 months | 3.9 | 4.0 | 1.1–8.3 |
| Tympanosclerosis | 7 | 31.7 | 7.2–64.3 |
| Granulation | 9 | 4.2 | 0–12.0 |
| Chronic perforation | 0.3 | 2.2 | 0–12.3 |
| Cholesteatoma | 0 | 0.8 | 0–6.5 |
| Tube displacement into middle ear | 0 | 0.5 | 0–1.3 |

effect of an indwelling tube. Studies on the outcome of TTs have seldom reported the ventilation time of the tubes, but used the term "tube retention time" or reported the tube extrusion time, which is, by definition, the time at which the entire medial flange of the tube is seen to be out of contact with the tympanic membrane (Moore 1990). This issue is understandable, because of the difficulty in defining whether a tube is occluded or extruded. This may, however, lead to over-appreciation of long-lasting TTs, despite the fact that often the long-lasting tubes are occluded during the last months of their stay in the tympanic membrane, when they, being foreign objects, promote only sequelae.

It is obvious that a certain interval exists between the end of successful ventilation time and tube extrusion. In this prospective study, this interval varied between 0 and 8.5 months (unpublished data). A tube extrusion was defined as occurring when the tube inner flange was visible over the tympanic membrane, and tympanometry resulted in an ECV under 1 $cm^3$. It is possible that this definition leads to overestimation of the number of tube occlusions, which in this material was 37%, because some tympanic membrane healing can happen under a TT, allowing normal movement by the tympanic membrane even before the tube has actually extruded.

A high number of occlusions in titanium TTs (25%) was also found by Handler et al. (1988). This, together with the results of this thesis, shows that overall occlusion rate may be higher with titanium tubes or that occlusion and partial extrusion are particularly difficult to differentiate for titanium tubes, due to the very thin flanges that may remain inside the tympanic membrane during the healing process. Based on the results of this thesis, it is obvious that the large variation in number of tube occlusions in previous studies (0%—36%) is at least partly related to these problems in defining tube occlusion. The difference in number of early tube occlusions between HSA-coated and uncoated tubes in this prospective study is at least real, not the result of a difference in diagnosing extrusion and tube occlusion between coated and uncoated tubes. The reason is that at the 3-month visit, the number of tube extrusions (7) was the same for both tube types, but the number of tube occlusions (12) in HSA-coated tubes was half that for uncoated tubes (24). Moreover, the percentage of occluded tubes at the 3-month visit, 7% for HSA-coated and 14% for uncoated tubes, is similar to the percentage reported earlier for Teflon tubes (10.5%) (Jamal 1995).

Because the major benefits of the HSA coating were predicted to be evident during the first months of follow-up, the randomized follow-up was ended at the 9-month visit. During that time, only 25% of the tubes extruded, so some of the postoperative findings, such as average ventilation time and changes in tympanic membrane, are underestimated and some, such as need for re-tympanostomy, cannot be presented at all.

These results show that significantly fewer early tube occlusions occur in HSA-coated tubes, and—especially in cases with excessive blood contact with the tube during surgery—tube occlusions are fewer, leading to prolonged ventilation time with HSA-coated tubes. No adverse effects of albumin coating were shown in this or earlier studies. These results constitute one step forward in the search for the optimal tympanostomy tube.

# 7
# CONCLUSIONS

1. A TT coated with HSA can resist adhesion of fibronectin, the most abundant adhesive glycoprotein in exudates.

2. HSA will produce a smooth coating attached firmly to the TT surface. Resistance to fibronectin adhesion achieved with HSA coating is durable in an 8-month durability test.

3. HSA-coated titanium plates are resistant *in vitro* to adherence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, bacteria typical of chronic implant-derived infections such as chronic otorrhea in a tympanostomized ear.

4. In a clinical trial, HSA-coated TTs showed half the number of early tube occlusions as did uncoated TTs. Furthermore, among patients with excessive perioperative bleeding, ventilation time for HSA-coated tubes was 31% longer than for uncoated tubes.

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