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OTOLOGY

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Titanium as a biomaterial for ossicular replacement: results after implantation in the middle ear of the rabbit

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Abstract The middle ear poses unique challenges when finding suitable materials for ossicular reconstruction, primarily because of its link to the external environment via the eustachian tube and, hence, its greater exposure to infectious agents. In this study, the biocompatability of titanium was examined in the middle ear of rabbits by using light and scanning electron microscopy. Implants were placed as middle ear prostheses or as free implants. These were inspected at 28 days, 84 days, 168 days, 336 days and 504 days following implantation for mucosal coverage, percent epithelization and any sign of foreign-body reaction. After 28 days, the prostheses were covered by regular mucosa. Although a majority of the free implants took up to 336 days for complete epithelialization, some of the free implants were not epithelialized even at day 504. There were no inflammatory cells observed on the surface of the material, nor were unusual amounts of fibrous tissue seen. In addition, the titanium material exhibited an affinity toward bone. The results of this animal experiment indicate that titanium is a favorable material for ossicular replacement prostheses.

Key words Hearing loss · Biomaterial · Ossicular replacements · Middle ear titanium · Animal experiments

Introduction

Reconstruction of the ossicular chain due to chronic ear disease has made it necessary to assess many different types of materials for ossicular replacement. However, the middle ear poses unique challenges when identifying suitable materials for prosthetic development. This is due in part to the connection of the middle ear to the external environment via the eustachian tube, thus setting the stage for contamination during otitis media or upper respiratory tract infections. Autologous ossicles often cannot be used for reconstruction due to underlying disease, while homografts can possibly transmit such infectious diseases as hepatitis and HIV. For these reasons biomaterials are often examined as an option for reconstruction.

Biomaterials that have been used in middle ear reconstruction include different types of ceramics (aluminum oxide, bioglasses, hydroxyapatite), carbon materials, glass ionomer cement and such synthetic materials as a high-density polyethylene sponge [HDTS (Plastipore)] and polytetra fluorethylene-vitrous carbon (Proplast). Metals have also been used, primarily in composite prostheses with platinum, stainless steel, tantalum or gold.

Titanium has been used as a prosthetic material for many years in craniofacial and orthopedic surgery, indicating its potential utility in middle ear reconstruction. Its appealing qualities include corrosion stability and non-toxicity. Because the special middle ear environment makes it necessary to perform biocompatability trials, even if materials are well tolerated elsewhere, this long-term preclinical study was performed to demonstrate the biocompatibility of titanium in the middle ear.

Materials and methods

Commercially pure titanium (purity 99.427%, Leibinger, Freiburg, Germany) was used as the biomaterial. This was fashioned into pins measuring 4 mm in length and 0.4 mm in diameter, with a surface roughness represented by 15-μm longitudinal grooves.

A total of 36 healthy New Zealand female rabbits (Charles River Germany, Sulzfeld, Germany) were housed one per cage and given normal food and water until they weighed 2–3 kg (6 months old). At this time, the animals were anesthetized with lidoacaine-HCl + parahydroxy-benzoicacidmethylester (Rompun, Bayer, Leverkusen, Germany) and ketamine-hydrochloride (Ketanest, Parke-Davis, Berlin, Germany). A left tympanomeatal flap was then raised under sterile conditions, the lateral attic wall was partially removed, and the incus and head of the malleus were dissected away. The titanium pins were next interposed, primarily as total ossicular replacement prostheses (TORP) between the stapes footplate and the handle of the malleus. A second pin, serving as a free implant, was placed in the bulla away from the ossicular chain.

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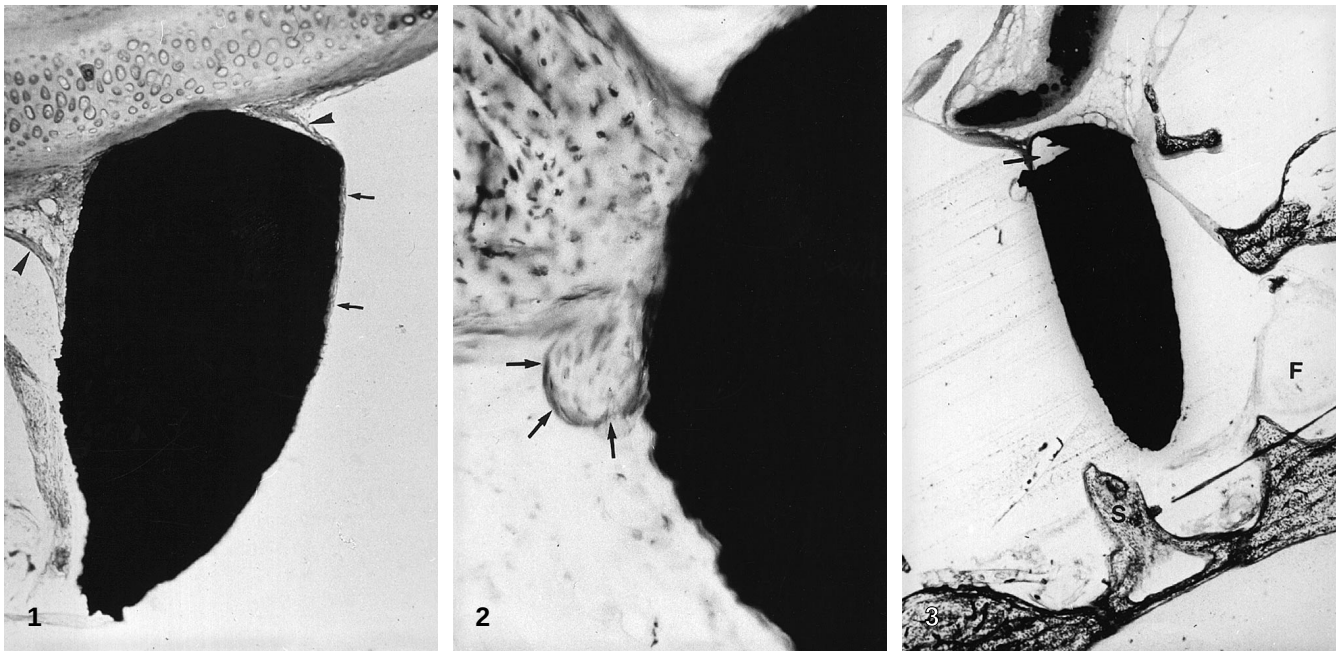


Fig. 1 Fixation by fibers of connective tissue (*arrowheads*) in an 84-day specimen. Cartilage has been used to reconstruct the tympanic membrane. Mucosal coverage (*arrows*) is present on the prosthesis. Giemsa, × 72

Fig. 2 Osteoid formation (*arrows*), bone contact of the implant (prosthesis) in a 336-day specimen. Giemsa, × 299

Fig. 3 Prosthesis after 504 days of implantation. The tympanic membrane has been reconstructed by cartilage. The figure shows the cutting artifact (*arrow*), stapes (*s*) and facial nerve (*f*). Giemsa, × 209

The retraction defect of the tympanic membrane was then closed by an underlay technique using perichondrium and cartilage. A sham-operated animal was prepared as a control for each animal group except for the time period at the termination of the study (day 504). In addition, the unoperated ear of each animal served as a negative control.

At the time of sacrifice on days 28 (n = 8), 84 (n = 7), 168 (n = 7), 336 (n = 8), or 504 (n = 6) post-surgery, temporal bones were removed and fixed in formalin. The specimens were embedded in epoxy resin, and histologic slides were cut by a Leitz saw (Leitz, Wetzlar, Germany) to a thickness of 30–80 µm (average, 50 µm). Staining was performed using the Giemsa technique for light microscopy. One specimen from each time point was also prepared for scanning electron microscopy.

Histologic analysis was performed to determine the presence or absence of tissue reactions toward the titanium prostheses or free implants. Because the development of epithelium on the surface of the pin was of major interest, measurements of the thickness of the mucosa were done at the time of death. In addition, percent epithelialization was determined at each time period by dividing the circumference of the epithelialized area by the total circumference of the implant.

Results

Macroscopic findings

The majority of animals killed at day 28 had open meatuses and showed more cerumen deposition in the operated ear. Stenosis of the ear canal was apparent in 2 of these animals and slight inflammation of the middle ear was observed in all animals. The tympanic membrane was closed by 28 days in all animals examined (n = 7). Findings were similar at day 84, but the amount of cerumen in the operated ears had increased.

By day 168, ceruminal debris was observed in four of the six animals examined, while three of these with extensive detritus also showed cholesteatoma-like findings, with the eardrum pushed medially and thinned and debris entering a ruptured membrane. By 336 days, the eardrum of all animals examined (n = 7) was thin and without inflammation. Stenosis and detritus of the ear canal were remarkable in three of the seven specimens examined at day 336, with one tympanum containing cholesteatoma-like debris. In cases of ceruminal masses, the tympanic membrane was forced medially. At 504 days, two of the six animals showed ceruminal masses.

Light microscopy

The surface of the prostheses were completely covered by epithelium by day 28, and the mucosa appeared granulated. There were no inflammatory cells on the surface, although granulation tissue and free inflammatory cells and macrophages could be seen in the tympanum. Several expanded vessels were noticed in the tissue of the cavity wall. The macrophages were fused to giant cells in a few cases (2/6). Tissue adjacent to the free implants looked normal and showed no inflammatory response. However, mucosa could be seen on the surface of the free implants in only a few cases.

By day 84, there was no difference between the mucosa covering the prostheses and the mucosa in the tympanum in all specimens examined (n = 5), and vascularization of the tympanum was unremarkable. The prosthe-

ses were fixed by strong fibers of connective tissue toward the tympanic membrane (Fig. 1), and in the oval niche between the implant and the residues of the stapes crura. The fibers were strongest at the edges of the biomaterial. Some of the free implants were epithelialized, but others showed no mucosal covering. There was one case of a cholesteatoma-like finding in an 84-day specimen.

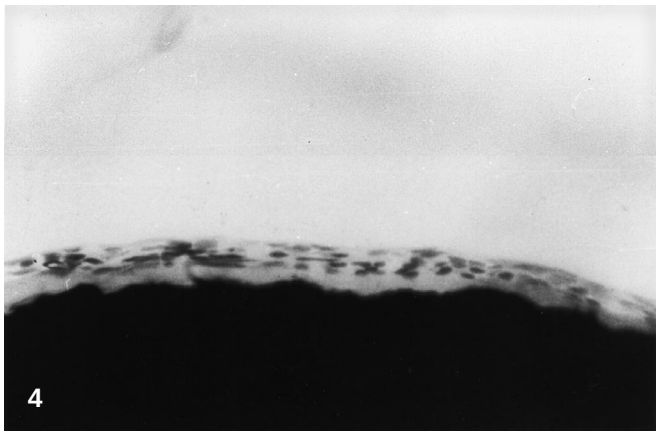
By 168 days, the prostheses in all animals examined (n = 5) were covered totally by mucosa, and the free implants exhibited increasing epithelial coverage. Some specimens showed narrow contact between the prosthesis and bone, sometimes without any layer of fibrous tissue, and growth of new bone close to the implant was observed. By 336 days, some of the prostheses were embedded in loosely connective tissue. No macrophages or giant cells could be found upon the surface of either the prosthesis or free implant. One specimen showed close bony contact, and another showed bony growth toward the surface (Fig. 2). The mucosa of the tympanum was without irritation, and only a few vessels could be noticed.

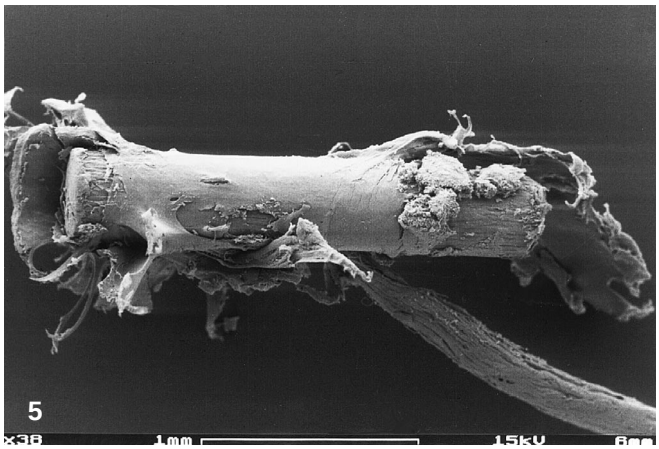
At 504 days, the cartilage of the reconstructed eardrum appeared to be unaffected by contact with the prosthesis (Fig. 3). The epithelium looked normal and the connective tissue was free of foreign-body cells (Fig. 4). Perineural or neural tissue showed no visible reaction in cases of contact with the facial nerve (with absence of a bony cover considered normal for the rabbit). Two of the five animals examined showed cholesteatoma-like findings, with leukocyte infiltration in the trapped corners of the bulla. However, free implants were also covered by normal mucosa at this time point.

Scanning electron microscopy

At 28 days, scanning electron microscopy revealed a normal tympanum, with the prosthesis covered by fibrous tissue and mucosa (Fig. 5). The epithelium showed polygonal squamous cells, normally found in the middle ear, covered by microvilli (Fig. 6). Only a few fibroblast-like cells were visible on the free implants. By day 84, no inflammatory cells could be seen, and the prostheses were covered by normal middle ear mucosa. Subepithelial fibrous tissue was fixed to the free implant in the tympanum.

Animals killed at day 168 and day 336 showed complete epithelialization of the titanium pins. Squamous epithelium cells were the only cells found on the biomaterial at 336 days, and no ciliated cells or goblet cells were observed. Adjacent regions of the control animal exhibited the same type of mucosa. By 504 days, the tympanum and prosthesis were covered by a smooth, microvilli-bearing epithelium, with equal coverage of both the free implants and the prostheses. There were no signs of abundant fibrous tissue around the biomaterials.





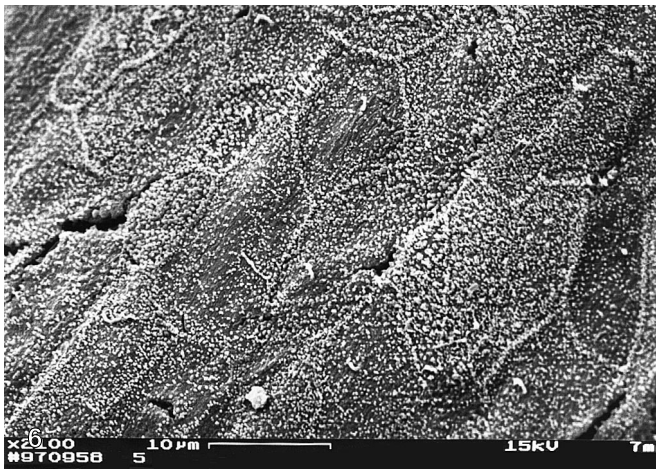


Fig. 4 A regular epithelium can be seen covering the prosthesis surface at 504 days. Giemsa, × 554

Fig. 5 Prosthesis has been removed from the middle ear after 28 days and is covered with connective tissue and epithelium. SEM, × 38

Fig. 6 Normal epithelium of polygonal squamous cells can be seen covering a prosthesis at 28 days. SEM, × 2100

Mucosal thickness and percent epithelialization

By day 28 the thickness of the mucosa averaged 36 µm. This measured 13 µm at 84 days and averaged 14 µm for

the remainder of the time. All prostheses were covered by mucosa at day 28. Percent epithelialization of the free implants was 25% after 28 days and was completed by 336 days post-implantation in most of the animals studied. However, at 504 days there were still some free implants that were not epithelialized.

Controls

The unoperated ears of each animal showed no visible disorders. All ears of the sham-operated controls contained significant amounts of cerumen, while one animal had developed a cholesteatoma in its outer ear canal.

Discussion

Before an alloplastic material can be used in the middle ear space, its reactions towards the surrounding tissue and special environment of the middle ear need to be examined carefuly. An important consideration is epithelialization of an implant material. In this investigation, there was a remarkable difference in mucosal development between the prostheses and the free implants. While the prostheses showed total epithelial coverage after 28 days of implantation, the mucosal coverage of the free implants was only 25% at this time. This difference is probably due to mucosal injury during insertion of the prosthesis. An injury is a strong stimulus for activating local growth factors to begin wound healing and involves the outgrowth of fibroblasts and epithelial cells. In contrast to prosthesis placement, the insertion of the free implant did not lead to mechanical injury of the mucosa. Thus, it took up to 1 year and, in some cases, even longer for the mucosa to cover the surface of the free implants. However, that the free implants were covered by normal middle ear mucosa was considered to be a sign of major biocompatibility. The thickness of the mucosa was greatest at day 28. After 84 days (approximately 3 months), it returned to normal values for middle ear mucosa. The high value obtained after 4 weeks was believed to be due to post-operative healing [11].

Histologic criteria for judging the biocompatibility of alloplastic materials are the amount of fibrous tissue present, the number and distribution of round cells and giant cells, and the vascular state of tissues adjacent to and surrounding the implant [9]. In our study, round cell infiltration was prominent at day 28. This was considered a normal condition of the wound-healing process, during which macrophages and giant cells remove cellular debris [11]. Inflammatory cells were also found at later times in animals in cases of secondary inflammation. However, no specimen exhibited round cells directly upon the surface of the implant material.

An obvious finding in the New Zealand white rabbit were changes in the outer ear canal after surgery. In some cases there was postoperative stenosis, while other animals displayed open canals. A failure to properly transport cerumen may have been the reason for the cerumen retention observed. Ear canal disorders with high amounts of ear canal detritus led to medialization of the eardrum, causing poor or no aeration of some compartments. Inflammatory cells, macrophages, and giant cells were seen in these areas. The ceruminal masses thinned the eardrum, which then ruptured to cause debris to be scattered in the tympanum. Thus, cholesteatoma-like findings were observed. However, such findings were only observed in the presence of ceruminal masses.

Other biomaterials that have been tested in the middle ear have shown varying amounts of inflammatory cells after implantation. The bioglass Ceravital is a bioactive calcium-silicon ceramic and is known to biodegrade, particularly in the infected middle ear, showing giant cells in the fibrous tissue surrounding the biomaterial [32]. This has resulted in a major loss of mechanical stability [33, 34]. Although aluminum oxide, a bioinert ceramic, has been used in middle ear surgery with success [22], animal studies have demonstrated macrophages and giant cells on the surface of this biomaterial during the primary post-operative period [20, 22, 23], indicating signs of potential host injection.

Because of its similarity to human bone, hydroxyapatite has been recognized as an excellent ossicular replacement material [14, 15, 39]. Its biocompatibility is assumed to be higher than that of natural dentin or dental enamel [5]. However, biodegradation has been observed in experimental studies [10], with macrophages and giant cells displayed in the rat middle ear after implantation [6, 16, 17]. Degradation was recorded at a rate of 15 µm per year [17]. Animal studies of two other polymers tested as middle ear implants, Plastipore and Proplast, showed enormous amounts of giant cells on their surfaces, and these resulted in extruded prostheses [2, 8, 9, 13, 25, 27] and great amounts of fibrous tissue [7, 24, 35, 38] after human middle ear surgery.

Carbon has also been examined as an ossicular replacement material, but animal studies showed marked foreign body reaction [21], with histology revealing fibrous encapsulation and giant cell formation [26]. In addition, carbon implants may be metabolized by certain microorganisms [4]. More recently, ionomeric cement was introduced in middle ear surgery [3, 12, 28, 29]. In animal studies, biodegradation could not be assessed after 336 days of implantation [11]. However, long-term histological results showed cellular signs of degradation and zones of loose material, lacunae, and giant cells were observed (after 5 years of implantation in the human middle ear) [18].

Metallic implants, such as Teflon wire for stapes surgery, are often used as combination prostheses. Gold has been used as biomaterial for stapes prostheses as well as for partial and total ossicular replacement prostheses [19, 31, 36]. However, the bioinertness of gold has not been proven histologically. Giant cells have also been seen close to the surface of gold implants removed from human middle ears [18].

Titanium has been used as a combination prosthesis with gold [30], with a clinical study reporting favorable

results in human middle ear surgery [37]. The affinity of titanium toward bone, known as osseointegration in bone-anchored devices [1], seems to occur in a similar way in the middle ear. Bone growth toward the prosthesis was recognized in our own experiments as well as clinically [37]. This could lead to problems in revision surgery or in bone connection of the prosthesis to the fallopian canal in the human middle ear, which could cause fixation of the ossicles and subsequent hearing loss. Unfortunately, osseointegration could not be investigated in the current study because the facial nerve of the rabbit has no complete bone cover over the oval window niche. However, the incidence of bone growth toward titanium should not be any higher than that toward autologous or homologous ossicles.

In conculsion, our present investigation demonstrates that titanium can be used successfully as a biomaterial in middle ear surgery. The excellent coverage by normal middle ear mucosa and the lack of macrophages and giant cells on the material's surface are histological signs of good acceptance of titanium in the middle ear of the rabbit. Even in cases of infected middle ear compartments, there was no sign of degradation or foreign-body reaction. Further studies will require audiologic testing to determine the true effectiveness of the titanium alloplast in the reconstruction of effective sound conduction.

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