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Efficacy of nanoporous silica coatings on middle ear prostheses as a delivery system for antibiotics: An animal study in rabbits

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ABSTRACT

Nanoporous silica layers are able to host molecules and release them over a certain period of time. These local drug delivery systems for antibiotics could be a new approach in the treatment of chronic otitis media. The aim of this study was to examine the efficacy of nanoporous silica coatings on middle ear prostheses as a delivery system for antibiotics in vivo. *Pseudomonas aeruginosa* was inoculated into the middle ear of rabbits to induce an otitis media. The control group received coated Bioverit®II implants without antibiotics. Coated prostheses with loaded ciprofloxacin were implanted into the middle ears of the study group. After 1 week, the rabbits were sacrificed. The clinical examination as well as the microbiological and histological examinations of organs and middle ear irrigation revealed clear differences between the two groups. *P. aeruginosa* was detected in every middle ear of the control group and was almost completely eliminated in the study group. Organ examinations revealed the presence of *P. aeruginosa* in the control group and a prevention of a bacterial spread in the study group. The nanoporous silica layer as antibiotic delivery system showed convincing efficacy in induced pseudomonal otitis media in the rabbit.

1. Introduction

The treatment of chronic otitis media (COM) is often complicated by recurring bacterial infections, which disturb the healing process, lead to persistent otorrhea and might even induce meningitis, brain or mastoid abscess [1–3]. Even after surgery aimed at the eradication of the infection and despite major progress in chemotherapy, microorganisms can persist and reproduce. Besides, the connection between the oral cavity and the middle ear through the Eustachian tube enables bacterial colonization, which is hard to avoid.

In many cases of COM the ossicular chain is destroyed and reconstruction is necessary. Microbial biofilms can form on freshly implanted material surfaces and impair the performance of the prostheses [4]. If the implants are infected or complications like extrusion occur, they have to be removed. An important factor for a successful implantation is a fast integration of the prostheses. Therefore, tissue cells of the organism have to cover the implant before bacteria adhere to the surface, which may lead to implant rejection [5,6]. There are several approaches to avoid bacterial adhesion and reproduction on medical devices. One possibility is the application of a material with mild antibacterial activity, such as ionomer cement, bioactive class ceramic or hydroxyapatite. For these materials, the antibacterial efficacy differs depending on the bacterium species [7]. Also bioceramics like Bioverit®II or Al_2O_3 ceramic show a slight antibacterial effect against Gram-negative bacteria, but complete elimination is not achieved. In contrast, glassy carbon serves as a source of nutrition for bacteria so that this material is unsuitable for ossicular reconstruction [8]. Another possibility is the surface modification of implants, for instance using special coatings with antibacterial activity. Berry et al. investigated phosphorylcholine-coated fluoroplastic tympanostomy tubes in comparison to silver-oxide impregnated and plain fluoroplastic tubes incubated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The coated tubes showed resistance to biofilm formation by both pathogens, whereas in plain tubes, growth of *P. aeruginosa* was detected, and in silver-oxide impregnated tubes, biofilms consisting of both microorganisms were detected [9].

An antibacterial effect can also be provided by a local drug delivery system releasing a drug at the critical site to kill bacteria in the surrounding area. Often, biodegradable polymers are used as a drug reservoir and release system [10]. In 2011, nanoporous

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silica materials were proposed as potential drug release systems [11]. In the beginning, investigations largely focused on in vitro applications and simple model drugs like ibuprofen, but more recent investigations have been conducted with a wide variety of drugs and biologically active molecules [12–15]. Nanoporous silica materials possess regular pores on the lower nanoscale (3–10 nm) and are also designated as mesoporous materials, according to the definition of the International Union of Applied Chemistry [16]. Their pore diameters are sufficiently large for the adsorption also of larger drug molecules. Nanoporous silica materials possess large pore volumes and high surface areas, allowing the absorption of large amounts of drugs, thus providing sufficient concentrations for local treatment. The surface of silica materials is reactive due to the presence of silanol groups. This allows for facile modification by silanization reactions and thus opens possibilities for enhancing the drug loading and for controlling the drug release [17,18]. The research in the biomedical application of this material has so far focused on nanoporous silica nanoparticles, whereas the device presented here is based on a coating, i.e. a continuous thin film, on a prosthesis. Using this implant-supported coating as a basis for drug delivery constitutes a direct pharmacological application form.

The nanoporous silica coatings we use have been evaluated in earlier studies [13,19–21] and have demonstrated excellent tolerance and tissue compatibility in cell culture and animal experiments, with one study in the middle ear of rabbits extending over a period of up to 360 days [19]. Many studies on nanoporous silica nanoparticles have proven their general biocompatibility [17,18,22–24], which has also been proven in some animal studies [23,25–27]. However, some studies have exhibited potentially harmful properties. In one investigation, nanoporous silica particles injected intraperitoneally or intravenously in a mouse model led to death or euthanasia [25]. A further investigation in a mouse model showed that subcutaneously placed nanoporous silica nanoparticles could increase tumor growth [28]. The FDA has recently approved the first in-human trial of nanoporous silica nanoparticles applied in the bloodstream [29].

The object of this study was to examine the application of a drug delivery system in reconstructive middle ear surgery. For this purpose, Bioverit®II middle ear prostheses were equipped with a nanoporous silica coating which was chemically modified to incorporate large amounts of ciprofloxacin [13]. These prostheses were implanted in the middle ear of rabbits, a useful animal model for middle ear surgery [30]. To demonstrate the antibacterial efficacy, otitis media was induced by *P. aeruginosa* application into the middle ear.

2. Materials and methods

2.1. Animals

For this study, 24 female New Zealand White rabbits were purchased from Charles River, Sulzfeld, Germany. The rabbits were reared up to a weight ranging from 3.2 to 4.2 kg in the Institute for Laboratory Animal Science and Central Animal Facility of Hannover Medical School, Germany. This study was conducted in accordance with the German law for animal protection and with the European Communities Council Directive 86/609/EEC for the protection of animals used for experimental purposes. All experiments were approved by the Local Institutional Animal Care and Research Advisory Committee and permitted by the local government (Ref.: 33.9-42502-04-09/1734).

2.2. Implants

In this study Bioverit®II prostheses from 3di GmbH (Jena, Germany) were used as the basic material for ossicular reconstruction. The implants consisted of a cylinder with a length of 2.5 mm and a diameter of 1 mm and at a right angle a plate with a diameter of ~3 mm (Fig. 1). The implants were coated using a dip-coating procedure to establish the nanoporous silica layer. In a fashion similar to the production of flat samples and as described in more detail elsewhere [13], coatings were produced by dip-coating the implants using a solution containing ethanol, water, hydrochloric acid, tetraethoxysilane as a silica source and a poly(ethylene glycol)-poly(propylene glycol)-block-co-polymer (from Sigma–Aldrich, similar to Pluronic P-123 from BASF) as the structure-directing agent. The implants were dipped by employing a DC Small Dip-Coater (NIMA, Coventry, UK) operated in a climate box at a constant humidity adjusted by 50 wt.% glucose solution. The coated implants were then left at constant humidity for 5 min. The samples were then dried at 60 °C for 30 min. This procedure was repeated twice. Finally, the samples were dried overnight and afterwards calcined at 415 °C for 4 h (rate of heating/cooling: 1 °C min^{-1}).

In order to improve the degree of loading with ciprofloxacin, the nanoporous coating was chemically modified so that its surface carried sulfonic acid groups. The surface silanol groups of the silica were first reacted with a mercaptosilane and then oxidized with hydrogen peroxide [13,31]. For this purpose, the implants were first cooled to 0 °C in 89 ml of dichloromethane before 12.76 ml of 3-mercaptotrimethoxysilane were added. The solution was gently stirred for 22 h without renewing the ice bath. The implants were then washed with dichloromethane and ethanol and dried at 100 °C for 5 h. Afterwards, they were placed in 100 ml of an aqueous hydrogen peroxide solution (30 wt.%) for 48 h at room temperature, followed by washing with water and absolute ethanol. Finally, the implants were dried at 60 °C for 2 h and cooled to room temperature.

The insertion of ciprofloxacin into the sulfonate-modified nanoporous coating was carried out in a 60 mM solution of the drug at pH 4 and at 37 °C for 3 days. At pH 4, the sulfonic acid groups are deprotonated and thus negatively charged whereas the molecules of the ciprofloxacin are protonated and thus positively charged, leading to a strong electrostatic attraction [13]. After the insertion, the implants were rinsed briefly with water to wash off any remaining solution at their outer surface. Afterwards the samples were dried for 2 h at room temperature at constant air humidity adjusted with 50 wt.% glucose. From the preceding work on ciprofloxacin release from glass slides coated once with modified nanoporous silica layers, it can be estimated that per 1 cm^2 of the macroscopic surface of the implant, ~2 μg of ciprofloxacin can be released, corresponding to ~0.32 μg of ciprofloxacin per implant (with a calculated implant surface of 0.16 cm^2).

We implanted two types of prostheses, which were allocated to two groups. In the control group (n = 7), Bioverit®II implants with a sulfonate-modified silica coating without antibiotic were applied. The study group (n = 7) consisted of implants with a ciprofloxacin-loaded modified nanoporous silica layer.

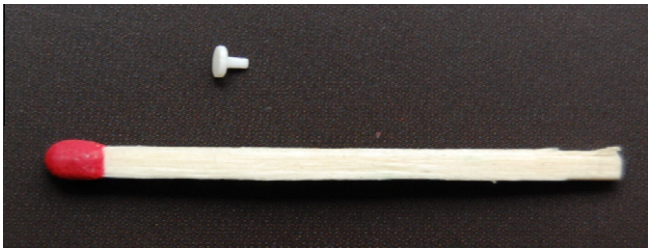

Fig. 1. Coated Bioverit® II implant.