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VALIDATION OF "TARGIS" AND "VECTRIS" OSSICULAR PROSTHESES IN GUINEA PIG MODEL

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ABSTRACT

The authors shared their results from the validation in a guinea pig model of a new ossicular prosthesis with Targis/Vectris System (Ivoclar, Lichtenstein). During the previous two years, the biocompatibility of teflon, gold, and ceramics was assessed. The biocompatibility of Targis and Vectris materials does not exist anymore as they are used in dental practice since 9 years. Targis is a ceramic optimized polymer of the family of ceromers. Vectris is a high-technology material replacing the metal substructure. These materials were inserted into a new medium, in the middle ear of a guinea pig. The experiments were carried out for different periods of time - 29 days, 70 days and 260 days. The ossicular prostheses were histologically examined. Targis and Vectris implanted retroauricularily into guinea pig' middle ear cavity did not show any inflammatory-reaction signs when contacting the skin of the external auditory channel. They proved to be well biologically tolerated even after 260 days after implantation.

Key words: ossicular prosthesis, Targis, Vectris, biocompatibility, histology, guinea pig

INTRODUCTION

The sounds from the ear canal can be transmitted to the inner ear in two ways: by the tympano-ossicular system (ossicular coupling) and by the direct acoustic stimulation of the oval and round windows (acoustic coupling) (17). When the ossicular chain is definitely damaged, it may be reconstructed using grafts or prostheses of different kind (6,7,19).

The idea of ossicular chain reconstruction (OCR) emerges and develops consequently from the creation of Wulstein's (22) and Zollner's (23) concept of tympanoplasty. Shea (21) moved from the concept of a graft to that of a bioprosthesis. He first began a successful series of OCR. Through the subsequent contributions of several authors (2,3,9,10,11,13) in the early 60s, a major pathway in the field of OCR was opened. In the next years, the advances in the new biomaterials permit the fabrication of commercialized ossicular prostheses of different kind, many of which remain still topical. The biomaterials used for OCR should possess a good biocompatibility and biostability (12). They must be well osteointegrated, with minimal risk of ankylosis (14). Their surface properties, and particularly their structural characteristics, critically influence the quality of the implant-recipient interface. Besides they should be easily processed and of stable shape. A proper sound transmission requires biomaterials of low mass and high hardness (16). In modern otosurgery, a large variety of biomaterials were made use of (1,4,5,6,7,8,15,20). None of them is, however, useful for any applications.

The aim of the present communication is to share the results from the validation in a guinea pig model of a new ossicular prosthesis with Targis/Vectris System dental polymer materials first used for ossiculoplasty. This system combines the advantages of the ceramics and plastics in one.

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MATERIAL AND METHODS

1. *Animal selection and initial surgery*

Fourteen adult guinea pigs of either sex, weighing between 350 and 500g, were selected and housed according to the Guide for the Care and Use of Laboratory Animals. Prior to surgery, the animals received an i. m. premedication bolus of 0,05mg/kg of atropine sulfate. Fifteen min later they were anesthesized using Xylazin 2% i. m. After 5min Ketamin i. m. was injected in a dose of 5mg/kg. The retroauricular areas were shaved and disinfected with Braunol. The implant was inserted in two sites using biomicroscope: in the middle ear cavity through the external auditory canal and in the *bulla mastoidea* using a mastoid trepanation. Each animal received antibiotics (Penicillin) during the operation. Two animals died during the initial surgery. The animals were returned to their cages and fed an unrestricted standard diet until the sacrifice. Each an-

imal received antibiotics three days after the operation (Penicillin).

2. *Second surgery*
Four animals underwent re-operation after four weeks. In each case, the second implant was inserted subdermally in the retroauricular area.

3. *Harvesting and processing*
Twelve animals were sacrificed either with an overdose of pentobarbital, or by exsanguination on day 29, 70 and 260 after initial surgery (Table 1).

Table 1. Distribution of animal ears according to the number of implantations

Day of sacrifice	Animals n	Implanted ear prostheses	Non-operated ears (controls)
29 day	4	4	1
70 day	2	2	1
260 day	6	6	4
Total	12	12	6

e implanted prosthesis was excised with some surround- ; tissues and fixed in a 10% formalin solution. Macro- pic pictures were taken from certain specimens.

Light microscopy
presentative specimens of harvested materials were cut. e biomaterial was explanted from the surrounding tis- s when possible. All these pieces were embedded in towax, 5-um sections were cut and stained with matoxylin-eosin, with AZAN, and by Masson's rome method.

RESULTS AND DISCUSSION

Ossicular prostheses explanted on day 29 after implantation
nty-nine days after implantation the ossicular prosthe- mplanted in the *bulla mastoidea* or *cavum tympani* ed to be covered by thin capsule in the process of rization. In this capsule, two layers can be distin- ied – an internal layer which contacts the prosthesis it- and an external one which covers the internal layer. rea of the capsule lying between the osseous wall and osthesis is at a more advanced stage of cicatrization in arison with that localized between the prosthesis and astoid or tympanic cavity, respectively.
xternal layer (towards the bone) of the area of the cap- ing between the osseous wall and the prosthesis con- f collagen fibers and fibroblasts (Fig. 1).

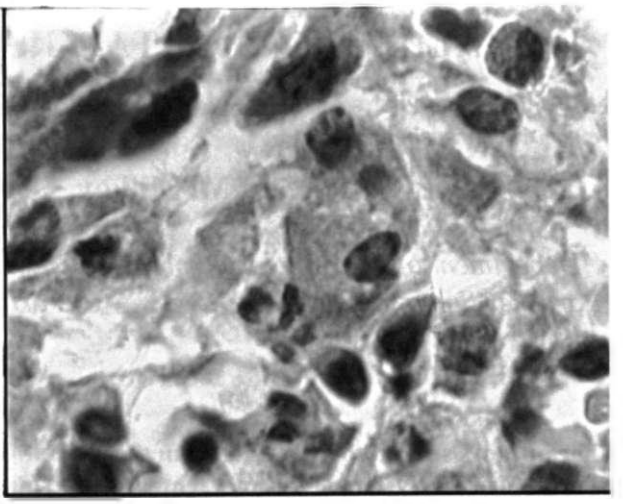

Fig. 1. Ossicular prosthesies explanted on day 29. The inner layer of a capsule disposed between the prosthesis and the mastoid/tympanic cavity, in which a small number of fibroblasts composing a fine grid are observed. Hematoxylin-eosin. Magn. x200.

Young cells with ovoid nuclei can be seen inwards and loosely linked with the connective fibres in which single inflammatory cells are ascertained in some animals.
The external layer has no sharp bordeline to the inner layer composed of small number of inflammatory cells in degeneration (necrotic polymorphonuclear cells along with groups of necrotic macrophages). The cells present with condensed chromatin and picnotic nuclei that suggests a possible apoptosis.

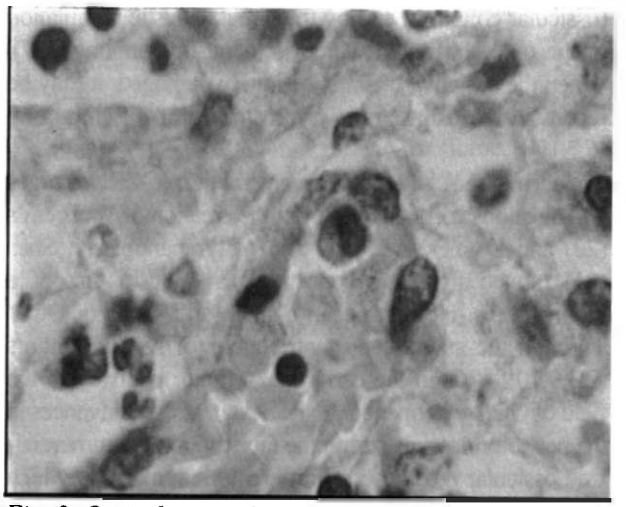

Fig. 2. Ossicular prosthesis explanted on day 29. In the inner layer of a capsule disposed between the prosthesis and the mastoid/tympanic cavity blood vessels with thin walls can be seen (neoangiogenesis). Hematoxylin-eosin. Magn. x 200

The external layer of a part of the capsule disposed between the prosthesis and the mastoid/tympanic cavity consists mainly of a numerous collagen fibers infiltrated with reduced number of inflammatory cells. In this layer, lymphocytes are most frequently observed but less monocytes and macrophages. However, in some animals a small quantity

of eosinophils and neutrophils can be seen. Heaps of dead and degenerated cells may be observed. In some regions there is a cicatrization in advanced stage. There are a few of inflammatory cells while the fibroblasts are more abundant. This external fibroblast layer is formed and grows from the periost of the mastoid cavity.

The inner layer of a capsule disposed between the prosthesis and the mastoid/tympanic cavity bordering the implant is of irregular thickness. It is composed of plasma proteins in process of cicatrization. In this layer there are few fibroblasts composing a fine grid.

The blood vessels with thin walls filled with erythrocytes can be seen, too (neoangiogenesis). The capsule is infiltrated by inflammatory cells the main part of which being a small group of macrophages. In some macrophages vacuoles (Fig. 2) in the cytoplasm and chromatin-like spokes can be seen.

According to their frequency the lymphocytes are the second cell population. Some of them are with picnotic nuclei. The polymorphonuclear cells (PMNC) or their residues are rarely observed and manifest symptoms suggesting apoptosis. Some cells of average size are disposed in the immediate vicinity of the material. Separate eosinophils with degenerative changes are found out, too (Fig. 3). In the lumen of the capsular neovessels eosinophils can be observed, too.

In some animals there are inflammatory cells such as PMNC, lymphocytes, monocytes and macrophages on the surface of the implant sections. Some cells manifest symptoms suggesting apoptosis.

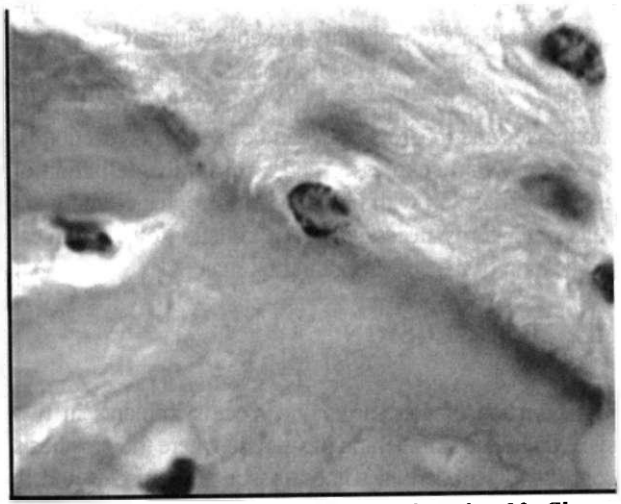

Fig. 3. Ossicular prosthesis explanted on day 29. Close contact between the implant and recipient capsule. The residues from PMNC with symptoms suggesting the apoptosis in the material are disposed. Hematoxylin-eosin. Magn. x 200

II. *Implants explanted on day 70 after implantation*

At the implantation of the prosthesis in the tympanic cavity for 70 days a fine connective tissue capsule was formed around the implant which consisted of 1 to 4 rows of fibroblasts (Fig 4). In one of the implanted animals separate or in-groups of about 10 inflammatory cells in some small regions around the capsule were observed. These were mainly macrophages and monocytes. In the other animal no inflammatory cells were found at all.

There was an impressively good contact between the conjunctive tissue capsule and the implant. There are no calcium deposits at all. Inside the pores of the implant some

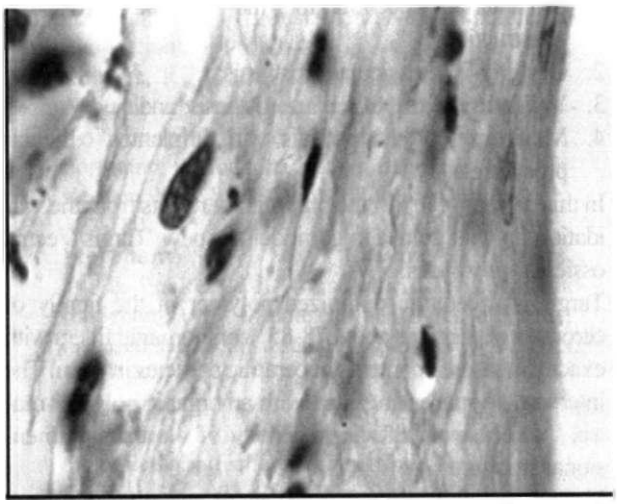

Fig. 4. Ossicular prosthesis explanted on day 260. Around the prostheses in cavum tympani, well-developed connective tissue capsule. Hematoxylin-eosin. Magn. x 200.

cells with picnotic nuclei were observed, probably macrophages. Their appearance suggests the presence of apoptosis.

III. *Implants explanted on day 260 after implantation*

Around the prostheses implanted for 260 days in *cavum tympani*, well-developed connective tissue capsule was found. In two of the implanted animals, single regions of the capsule were infiltrated with macrophages or with macrophages and lymphocytes. The tissues of external auditory meatus contacting the implant remain intact.

Around the prosthesis implanted in the subcutaneous tissue behind the cartilaginous part of the ear orifice, there is a well-shaped conjunctive tissue capsule. It is composed of several (up to 10) fascicles of collagen fibers and fibroblasts between them. In the capsule there are well-developed blood vessels (neoangiogenesis). At certain areas monocytes, macrophages, lymphocytes, and eosinophils are observed. At some small areas, between the material and the capsule, there are remainders of non-organized fibrinous mass containing macrophages. On the edge of the material and the remainder of non-organized fibrin, in the capsule may be found single macrophages. The tissues around the capsule are without changes. Hyperemia in some areas can be seen only. Separate lymphocytes are observed.

The capsule from the side of the muscle has the same structure in principle. On the edge of the capsule and the transversely furrowed musculature there are conjunctive tissue filaments penetrating between the fascicles of the muscle. The remaining parts of the muscle are without any changes.

The limited graft applications in the reconstructive auditory-chain surgery stimulated the search for a new prosthetic medical device. There are certain advances in the development of prosthesis fabrication designed for ossiculoplasty in recent years, indeed. Several questions are of paramount interest for solving this problem:

1. Biomaterial selection for the ossicular prosthesis construction;
2. Design of the ossicular prosthesis;
3. Validation of the ossicular prosthesis, and
4. Monitoring of the patients with implanted ossicular prostheses.

In this paper, we report the preliminary results from the validation in the guinea pig models a new Targis/Vectris ossicular prosthesis.

Targis is a ceramic optimized polymer of the family of ceromers. It contains about 78-85% of inorganic fillers with exactly measured contents of ceramic particles in them. The nterparticle spaces are filled with an organic polymer matrix that enhances the homogeneity of the three- dimensional structure.

Vectris is a high-technology material replacing the metal ubstructure. The material consists of numerous file-like lates layered one on the other and positioned in a common xis and forming a common fascicle. It combines the opportunity to maintain long-lasting loading on the account of s lower own weight in combination with long-lasting elascity and lower internal tensions.

n the combined application of these two materials, Vectris used for the fabrication of prosthesis substructure while argis is used for its coverage. In the literature available, usge of different animals for biomaterial validations has been ported. In a series of experiments on rats several omaterials for ossiculoplasty were implanted such as old, teflon, hydroxyapatite, ceromer, hydroxyapatite+ ain-steel and Silicon+stain-steel while in guinea pigs gold, flon, hydroxyapatite, and ceromer were used convincing in the good qualities of the guinea pig to serve as a model r testing the ossicular prostheses (18). In this experimen- investigation in guinea pigs model, some promising reIts have been obtained (18).

ter 29 day-long stay in the mastoid/tympanic cavity, the osthetic explants are covered by a fine capsule undergo- cicatrization. It consists of two layers. The cicatrization ocess is more advanced in the external layer presenting th outlined bundles of collagen and fibroblasts. This pros is less developed in the internal layer located closely to implant. There are scanty inflammatory cells most of ich characterized by degeneration. In both layers there newly formed blood vessels (neoangiogenesis). Based these features we could suppose that, similarly to any plants, this one analyzed in the present study causes, in onset, hyperemia and moderate inflammatory reaction idly replaced by a healing process resulting into the forion of a connective-tissue capsule around the implant, i. icatrization. These data are crucial taking into consider- n the general rule that the rapid capsule formation and helization of the implant substantially contribute to the prevention of microorganism seeding. In certain cases, macrophages, monocytes and lymphocytes could be established penetrating mainly in the pores on the external parts of the implant. These cells display degeneration signs.

It stresses that both Targis and Vectris implanted retroauricularily into the middle ear cavity of a guinea pig do not show any inflammatory-reaction signs when contacting the skin of the external auditory channel. A contamination of the material with bacterial cells occurred in two cases only. Having in mind the localization of the inflammation we allow us to assume that this material possesses bactericidal properties.

Around the prostheses implanted for 70 days in the mastoid/tympanal cavity a fine connective tissue capsule consisting of 1 to 4 rows of collagen bundles and fibroblasts is developed. Occasionally, in the capsule single monocytes and macrophages are seen. There exists a good contact between the connective tissue capsule and the implant itself. No calcium deposits could be detected. Cells with picnotic nuclei were observed, probably macrophages, inside the pores of the implant. Their appearance suggests the presence of apoptosis.

Around the prostheses implanted for 260 days the process of cicatrization of a capsule is well finalized. In single regions of the capsule only, infiltration by a few macrophages or by macrophages and lymphocytes was observed. No alterations in the tissues of external auditory meatus were observed.

Used in a specific environment such as mastoid/tympanic cavity and subdermally, Targis and Vectris prove to be well biologically tolerated, too, even after 260 days after implantation. Their biocompatibility presents with a well-formed capsule of collagen fibres, fibroblasts and newly formed blood vessels. In some animals, inflammatory cells like monocytes and macrophages can be seen. This reaction is, however, weakly expressed and far from the definition of alien-type reaction with histocytes, macrophages and giant cells obligatorily necessitating cancellation of the clinical application of the corresponding biomaterials.

CONCLUSION

The insertion of the Targis and Vectris implants in two sites, under the skin behind the auricle where the prosthesis contacts the subdermal adipose tissue and muscles and in the middle ear cavity and *bulla mastoidea* through two approaches (through the external auditory canal or through the mastoid cavity) enables the successful investigation of Targis and Vectris OCR biocompatibility (18).

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