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Evaluation of Soft Tissue Reaction to Corundum Ceramic Implants Infiltrated with Colloidal Silver

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Corundum ceramic is a biomaterial used as a bone graft substitute. Silver is a well known antiseptic substance with many practical, clinical applications.
Objectives. The aim of this study was to estimate soft tissue (*in vivo*) reaction to a new kind of ceramic implants. In our experiment, we examined the soft tissue reaction after implantation of corundum ceramic infiltrated with colloidal silver in the back muscles of 18 Wistar rats. The use of colloidal silver as a coating for the implant was designed to protect it against colonization by bacteria and the formation of bacterial biofilm.
Material and Methods. In our study, based on the experimental method, we performed implantation operations on 18 Wistar rats. We implanted 18 modified ceramic implants and, as a control group, 18 unmodified implants. As a follow up, we observed the animals operated upon, and did postoperative, autopsy and histopathological examinations 14, 30, 90 and 180 days after implantation.
Results. We didn't observe any pathological reactions and significant differences between the soft tissue reaction to the modified implants and the control group.
Conclusions. Lack of pathological reaction to the modified implants in the living organism is the proof of their biocompatibility. This is, of course, the first step on the long path to introduce a new kind of biocompatible ceramic implant with antiseptic cottage. Our experiment has an only introductory character and we plan to perform other, more specific, tests of this new kind of implant (**Adv Clin Exp Med 2016, 25, 1, 129–133**).

Key words: biomaterials, corundum ceramic, colloidal silver, implants.

Corundum ceramic is known as a biomaterial with a wide spectrum of medical use [1, 8]. As any other biomaterial, it may be a base for bacterial biofilm formation after implantation into a living organism. Repeatedly, it has lead to infection and failure of the medical procedure [12, 13]. Covering of the implant's surface by a layer of an antibacterial substance is widely promoted and used for the protection of temporary and final implants [4, 10, 19]. Numerous attempts confirm the effectiveness of using antibiotics for this purpose, but also reveal the limits of the method for some antiseptics [3, 5, 11, 15, 17]. There is no one universal antibiotic that protects implants against colonization by bacterial streams, and this is the reason for the search for new solutions, using different substances with confirmed antibacterial activity [2, 9, 14, 13]. Colloidal silver is a substance of confirmed antibacterial activity [6, 20, 21]. The possibility of using colloidal silver for ceramic implant protection requires testing the tissue reaction to such a modified implant. The aim of our experiment

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was to evaluate the soft tissue reaction to corundum ceramic implants infiltrated with colloidal silver after implantation into rat back muscles.

Material and Methods

We performed our experiment in the Department of Experimental Surgery and Biomaterials Research of Wroclaw Medical University with the consent of the Local Ethics Committee (resolution 30/2007 30.06.2007). Implants made of corundum ceramic were infiltrated with colloidal silver and prepared in the Institute of Electrical Engineering in Wrocław in the Department of Ceramics and Bioplastics. Samples with a cylindrical shape 4 mm in length and 2 mm in diameter were made from ceramic mass AL70E which contained 70% Al2O3. The ceramic mass was dried at 200°C for 6 h and then the dried samples were fired at 1550 degrees for 1 h. A solution of colloidal was obtained in the detonation chamber with the use of electric pulses. It was obtained from silver wire of 4N purity and 0.5 mm in diameter.

The voltage used in the process was 8.5 kV, the energy of the discharge was 100 J, and the amperage was 19 kA.

The ceramic implants were infiltrated in a solution of colloidal silver with 60 μg/mL concentration. The samples were immersed in the solution of colloidal silver for 30 min and then infiltrated in a vacuum chamber. The infiltration process was carried out at a pressure of 50 hPa for 20 min. The infiltrated implants were dried in the open air for 24 h, then in 70°C for 4 h and in 110°C for 2 h to remove the water from the samples. The infiltration process was performed twice. We prepared 18 implants with colloidal silver and 18 implants without silver as a control group.

The control samples were sterilized at 190°C for 3 h. The infiltrated samples were placed in a sterile container filled with the colloidal silver solution.

Course of Experiment

Sample Implantation

The properly prepared samples were implanted into the back muscles of 18 Wistar strain rats from inbreeding (age from 4 to 6 months and average weight 200 g). As an anesthesia, we used ketamine 75–100 mg/kg and xylazine 10 mg/kg, injected intraperitoneally. After preparing the operation field, we made a 2 cm length paraspinal skin incision. We prepared 2 pockets in the back muscles. On the left side we placed the samples with colloidal silver and on the right side samples from the control group. We used 2 single absorbable sutures to close the muscle pockets. The skin wounds were closed with the use of continuous nonabsorbable sutures (Fig. 1).

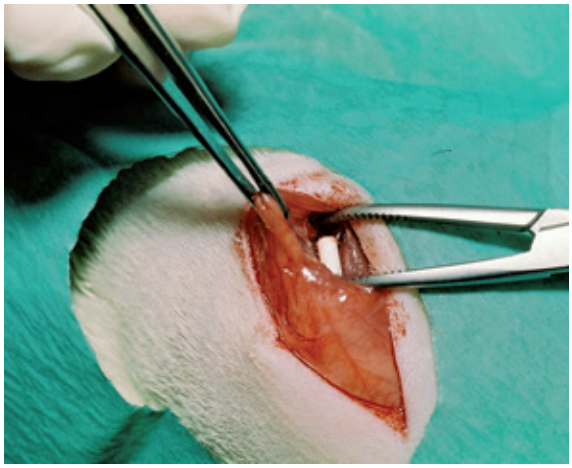

Fig. 1. Implantation of the sample into the back muscles of the rat

Postoperative Examinations

During the postoperative examinations, we evaluated the general state of the animals' health, their appetite, mobility and wound healing.

Autopsy Examinations

We performed the rats' autopsies 14, 30, 90 and 180 days after surgery. The procedure of euthanasia consisted of an intraperitoneal injection of pentobarbital (verbutal or morbital), and disruption of the spinal cord after cardiac arrest. This procedure was in accordance with the application to the Local Ethical Committee for permission to experiment on animals. During the autopsy, we evaluated postoperative scars, the appearance of the tissues that surrounded the implants, and the tissues which had direct contact with the implants. We also took sections of the thorax and abdominal cavity organs. During the autopsy, we took samples of the soft tissues which surrounded the implants.

Microscopic Examinations

All of the samples obtained were fixed in an 8% formaldehyde solution with calcium carbonate at room temperature for 3 days. Afterwards, we cut the muscles and soft tissue capsules around the implants to remove them. Samples of the soft tissue were dehydrated in a series of acetone at a temperature of 333°K for 90 min in 3 consecutive acetone

baths. Afterwards, the samples were immersed in carboxylene and xylene.

Such prepared samples were impregnated in paraffin blocks. We evaluated the histological specimens stained with hematoxylin and eosin by light microscopy.

Results

Results of Postoperative Examinations

All animals survived the operation, and none of them died during the experiment. All observed animals during the whole experiment had normal motor activity, appetite and weight gain. The postoperative wounds healed without complication.

Results of Macroscopic Autopsy Examinations

During the autopsies 14 days after implantation, we observed a thickening of the fascia over the ceramic implants. We also saw extended dark-red blood vessels around the implants.

It was difficult to dissect the implants from the surrounding tissues (Fig. 2).

Thirty days after implantation, we observed a greater thickening of the fascia over the implants with colloidal silver (Fig. 3).

Ninety and 180 days after the operation, the soft tissues around implants looked similar. We observed a thin and bounded capsule around the ceramic. It was strongly connected to the muscles and the implants (Fig. 4). The autopsy of the internal organs in all of the examined animals did not show any pathological changes.

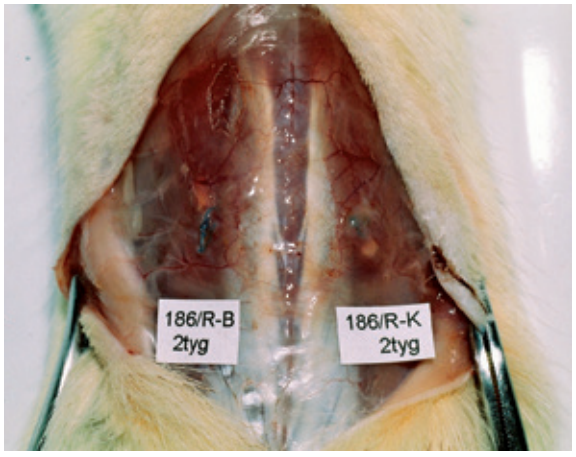

Fig. 2. View during autopsy 14 days after implantation of the sample

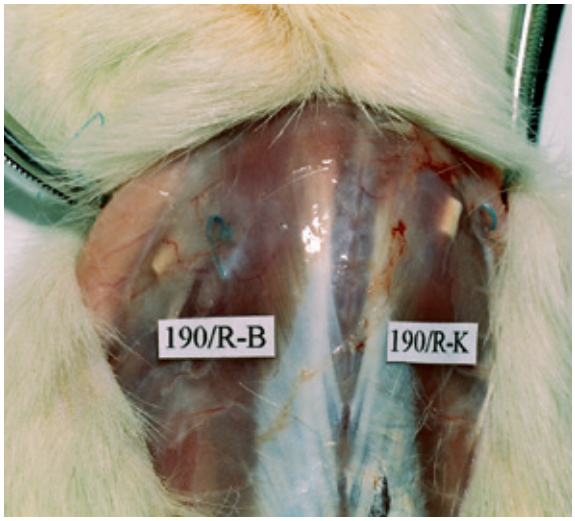

Fig. 3. View during autopsy 30 days after implantation of the sample

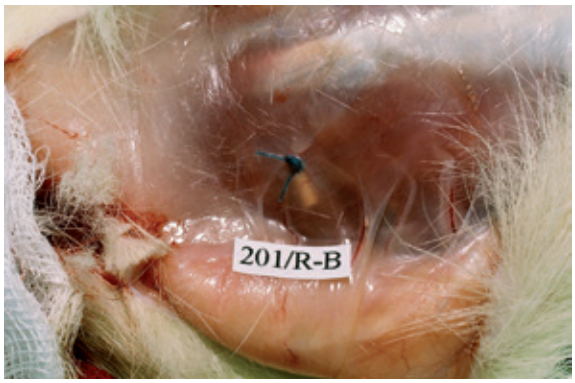

Fig. 4. View during autopsy 90 days after implantation of the sample

Results of Microscopic Examinations

In the histological specimens 14 days after implantation, we observed the space after the removed implant surrounded by connective tissue and normal, healthy striated muscles. The width of the connective tissue band ranged between 2 and 4 muscle fibers. The picture was the same in the examined and control group (Fig. 5).

Thirty days after implantation, the connective tissue around the samples had a double-layer structure. The layer which directly covered the implant had a more fibrous character. The width of this band was greater than the width of the muscle fiber. The second layer was made of loosely woven connective tissue (1–4 muscle fiber width) and was directly connected to muscle tissue (Fig. 6).

Ninety and 180 days after implantation, the picture was similar. The space after the removed implant was surrounded by a double-layer connective tissue band. The layer which was directly

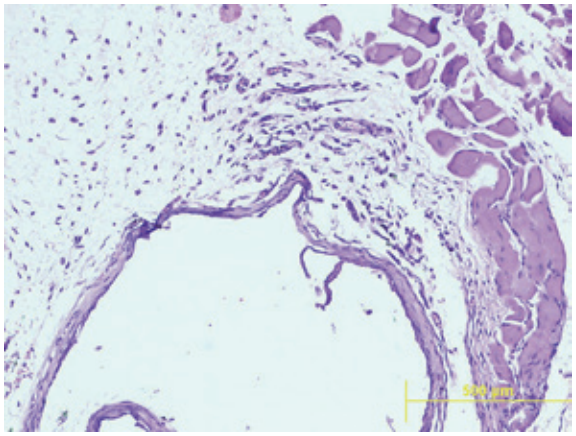

Fig. 5. Histological specimens 14 days after implantation with the space after the removed implant surrounded by connective tissue and normal, healthy striated muscles

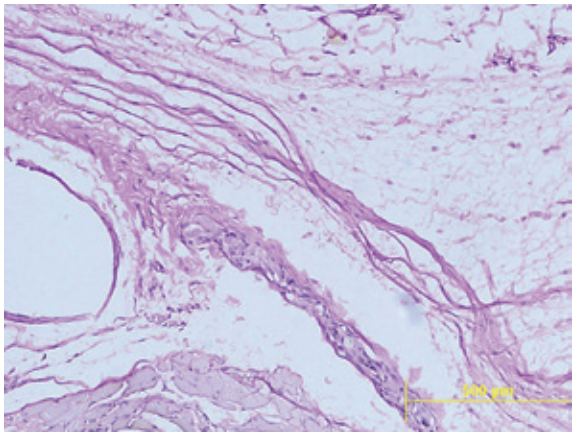

Fig. 6. Histological specimens 30 days after implantation, connective tissue around the samples with a double-layer structure

connected to the implants was compacted, fibrous and had a small number of cells. The superficial layer had a loosely woven structure (Fig. 7).

Discussion

We did not observe significant differences in the results of the macro- and microscopic examinations between the tested and control group. The reaction of the soft tissues was typical for a living organism's reaction to inorganic material implantation [7, 18]. As a result of the soft tissue reaction,

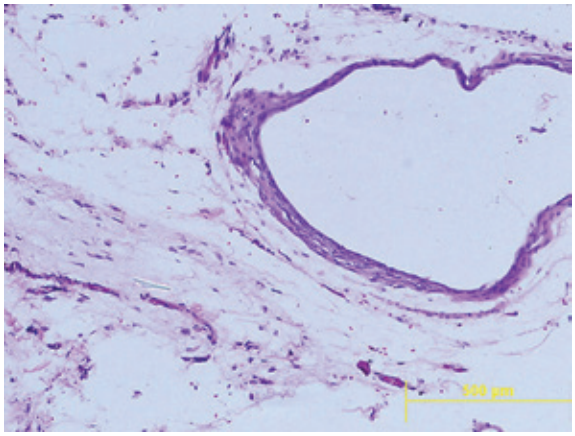

Fig. 7. Histological specimens 90 days after implantation. The space after the removed implant surrounded by double-layer connective tissue band. The layer which is directly connected to the implants is compacted, fibrous and has a small number of cells. The superficial layer has a loosely woven structure

we observed the formation of a connective tissue capsule, which was strongly connected to the implanted samples.

Our experiment had a preliminary character and was focused on the evaluation of soft tissue reaction to corundum ceramic implants infiltrated with colloidal silver. The next step will be the evaluation of bone tissue reaction to such modified implants. We will perform our experiment on rabbits. Only the combination of both experiments' results will allow us a full assessment of a living organism's reaction to our implants. We must remember the bilateral reaction between an organism and implants. As a result, we can observe the changes in tissues and implants. Therefore, in the future, an assessment of colloidal silver release and its biodegradation process is necessary. In additional microbiological tests, we also proved the bacteriostatic activity of colloidal silver [21]. The fact that there was no difference in the soft tissue reaction between the examined and control group may have a significant meaning for the possibility of therapeutic use of such modified implants.

In the face of the development of antibiotic resistance, different bacteriostatic substances may be useful in the prevention and healing of bacterial infections.

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