

Experimental Assessment of a New Type of Carbon-Coated ARTECOR® Vascular Prosthesis in Sheep

Jiří Podlaha¹, Martin Dvořák¹, Věra Žižková², Rudolf Dvořák³,
Radovan Kabeš³, Miroslav Jelinek⁴, Karel Veselý⁵

¹2nd Department of Surgery, Faculty of Medicine, Masaryk University, St. Anne's University Hospital Brno, Czech Republic

²Knitting Research Institute, Brno, Czech Republic

³Clinic of Ruminants Diseases, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

⁴Institute of Physics of the ASCR, Prague, Czech Republic

⁵Department of Pathology, Faculty Of Medicine, Masaryk University, St. Anne's University Hospital Brno, Czech Republic

Received April 16, 2008

Accepted November 12, 2008

Abstract

The aim of the study was to test and verify the characteristics of a new type of carbon-coated ARTECOR® vascular prosthesis developed at the Knitting Research Institute, a.s. Brno.

Eight healthy Merino sheep, aged between 2 and 3 years, were implanted four types (A, B, C with diamond-like carbon (DLC) coating and D as a control without DLC) of vascular prostheses. The site of implantation was the common carotid artery; the length of the implant was 7 cm. All sheep received antibiotics prophylactically in accordance with the theory of the so-called "protected coagulum". Doppler ultrasound examination was performed before finishing the operation to verify the patency of each prosthesis. During the study period the animals were closely observed. Prostheses were extirpated on day +/- 100 in 6 sheep and on day 182 in 2 sheep. Type B prosthesis showed better results according to its postoperative patency. The implant lumen was constantly 7 mm, whereas the use of other types resulted in lumen narrowing. Type B prosthesis has a deposition of DLC coating of a thickness of 20 nm with a high content of sp³ bonds (more diamond-like ones).

The experimental type B of prosthesis ARTECOR® appears to be the most successful of the tested prostheses (at the end of the study all B-type prostheses remained patent). This prosthesis appears to better satisfy the rheologic characteristics for healing.

Experimental work, vascular prosthesis, carbon coating, pre-clinical study

The development of vascular prosthesis tries to ensure the longest period of lumen patency possible. It is well known that the lumen of the prosthesis must be as smooth as possible in order to improve the patency, while the outer layer or surface of the prosthesis should be rough for better healing. In this case carbon-coating is thought to improve this parameter and to reduce body reactions by being inert (Sawyer 1987).

Each vascular prosthesis must comply with the following requirements:

- Surgical acceptance
- Security
- Long-term patency

The flow inside the prosthesis must be laminar and not turbulent (Podlaha et al. 1987). Vascular prostheses inside the body behave as foreign material and are very easily colonised by microorganisms.

Studies of the electrochemistry occurring at the blood synthetic interface of synthetic grafts and in normal blood vessels established that the inner surface of blood vessels are electronegative compared to their outer surfaces (Sharp 1998). This evidence suggested

Address for correspondence:

Jiří Podlaha,
2nd Department of Surgery, Faculty of Medicine
Masaryk University, St. Anne's University Hospital Brno
Pekařská 53, 659 01 Brno
Czech Republic

Phone: +420 543 182 467
Fax: +420 543 182 002
E-mail: jiri.podlaha@fnusa.cz
<http://www.vfu.cz/acta-vet/actavet.htm>

the theory that a negative surface prevents thrombosis while a positive surface promotes it (Sharp 1998). Carbon has an electronegative charge and has been found to act as a thrombosis deterrent (Sharp 1998). Carbon has been used to improve the thromboresistance of synthetic vascular prostheses and for this purpose, Dacron grafts have been coated with carbon (Ratto et al. 1988). In the presence of blood, a protein layer is formed, preventing the formation of blood clots at the carbon surface. For medical prostheses that are in contact with blood (heart valves, anastomotic sheets, stents, blood vessels, etc.) diamond-like carbon (DLC) coatings can thus be used (Hauert and Miller 2003).

The properties of DLC coatings depend strongly on the hydrogen content and the sp^3/sp^2 ratio which, in turn, depends on the deposition process and its parameters (Hauert and Miller 2003).

ARTECOR® vascular prostheses were subjected to biocompatibility tests; they complied with the requirements of non-toxicity, non-antigenic and non-carcinogenic properties, they showed permanent inertness; tests of haemolytic effect, cytotoxicity and pyrogenicity were performed with satisfactory results (Walter et al. 1991).

Other tests of biocompatibility such as allergisation, irritability, systemic toxicity, subchronic toxicity, and genotoxicity tests were performed with the RaK vascular prosthesis with collagen which has been manufactured for several years based on the approval No. 89/0313/00-IIB of SUKL (State Institute for Drug Control). The RaK prosthesis is manufactured of the same source materials as the ARTECOR® prosthesis (references a-i). The difference is only in the design of the prosthesis, lower density of knitted fabric (1). Carbon-coated ARTECOR® prostheses were delivered by the Institute of Physics of the Academy of Sciences of the Czech Republic.

Materials and Methods

The experimental animals used were eight Merino sheep, aged between 2 and 3 years (Enzler et al. 1994), free of infectious diseases. After clinical examination and tests they were moved to the experimental department of the Clinic of Ruminant Diseases of the University of Veterinary and Pharmaceutical Sciences Brno. The animals were quarantined that enabled their adaptation at the same time (Giardino et al. 1995). They were fed a standard feeding ration of meadow hay *ad libitum* with added concentrates and vitamin-mineral mixture. Their health condition was monitored every day.

Prior to the beginning of the experiment, clinical examination and detailed analysis of the milieu interieur were performed using biochemical and haematological laboratory tests.

The following indicators were determined in blood plasma: total protein, albumin, glucose, creatinine, urea, total bilirubin, aspartate amino transferase and gamma glutamyl transferase activities, and Na, K, Ca, Cl, and Mg concentrations. Complete blood count and differential count of leukocytes were determined in blood. Determination of the following blood coagulation tests was performed: thrombin time, fibrinogen, and prothrombin time. Examination results confirmed that the experimental animals were healthy, without any changes in the selected milieu interieur indicators. The same tests were performed before extirpation of the prosthesis.

Carbon-coated ARTECOR® prostheses were divided into four groups according to their properties and characteristics:

Group A - prosthesis with deposited DLC coatings of 150–200 nm thickness with a high content of sp^3 bonds (more diamond-like bonds)

Group B - prosthesis with deposited DLC coatings of about 20 nm thickness with a high content of sp^3 bonds (more diamond-like bonds)

Group C - prosthesis with deposited DLC coatings of 150–200 nm thickness with a low content of sp^3 bonds (more graphitic bonds)

Group D - control group prosthesis.

The study comprised two experimental phases.

In the 1st phase the convenience of the new type of vascular prosthesis was evaluated (Plate VIII, Fig. 1)

The Knitting Research Institute proposed to choose the most convenient ARTECOR® vascular prosthesis with carbon coating based on the results of pilot preclinical trials in sheep. In the first phase we intended to implant

A) 3 pieces of each type of the ARTECOR® C vascular prosthesis (types labelled A, B, C) and 3 pieces of vascular prosthesis without carbon (control group labelled D) for a time period of about 100 (95 to 104) days.

B) 1 piece of each type of the ARTECOR® C vascular prosthesis labelled A, B, C and one piece of the vascular

prosthesis without carbon (control group labelled D) for a time period of about 200 (182) days.

We supposed that a difference would be found among individual types of vascular prostheses with different carbon coating during the first phase. In that case the experiment would continue with the second phase. If no difference appeared, then the second phase would be omitted.

In the 2nd phase the chosen ARTECOR® vascular prosthesis with carbon would be subject to more extensive testing (10 prostheses of the experimental group and 10 prostheses of the control group).

Before the surgery, antibiotics were administered prophylactically in accordance with the theory of the so-called "protected coagulum". All the animals were anaesthetised after 8 h of fasting. The animals were not allowed to drink for the last 2 h before anaesthesia. The anaesthesiologic protocols for the first and second anaesthesias were identical. The sheep were premedicated with 0.2 mg/kg midazolam i.m. (Dormicum, Roche). Then a 16G intravenous catheter (Braunyle, B.Braun) was inserted into the saphenous vein with subsequent premedication with detomidine (Domosedan, Pfizer) at a dose of 10 µg/kg i.v. With the commencement of the detomidine effect, general anaesthesia was induced with an intravenous bolus of ketamine 5 mg/kg (Narkamon, Spofa). For facilitation of intubation, propofol (Propofol 1%, Fresenius Kabi) was administered at a dose of 0.5–1 mg/kg (until the effect). Then the sheep were intubated with an endotracheal cannula and positioned spinally and connected to an inhalation device. General anaesthesia was maintained using isoflurane (Forane, Abbott) with ET_{ISO} 1.3–1.4 volume percent. During anaesthesia, sheep were ventilated using CMV with a tidal volume of 6–10 ml/kg, PEEP up to 5 cm H₂O, and PIP up to 20 cm H₂O. During anaesthesia, vital functions were monitored (Datex S/5, Datex-Ohmeda). Continuous infusions of crystalloid solutions (Ringer's solution, Barter's solution) at 10 ml/kg/h were administered perioperatively. Mean arterial pressure was maintained at above 75 mm Hg. In case of its decrease below this value, continuous infusion of dobutamine (1–3 µg/kg/min) was administered.

After draping the animal in dorsal recumbency with disposable drapes, surgical exposure of tissue was performed to the right and left sides in front of the sternocleidomastoid muscle from a longitudinal incision above the trachea. Careful surgical exposure of the common carotid artery was performed; attention was focused on separation from the nerve plexus, with special attention to the n. vagus. After intravenous administration of heparin (ca 10 000 IU according to the animal's weight), the carotid artery was closed with vascular clamps. Subsequently a segment of ca 7 cm in length of the right common carotid artery and then of the left one were replaced by the prostheses. Suture material Prolene 6-0 with a 13 mm needle (W 8706) were used. Continuous suture was performed. Edges of at least 2 mm were taken both of the prosthesis and the artery. "End-to-end" anastomoses were performed. After suturing the prostheses and careful haemostasis, the platysma muscle and skin were sutured. Heparin was not neutralized with protamine sulphate and anticoagulation therapy was not administered after the surgery.

After the surgery, the inhalation device was disconnected and the sheep were moved to the post-operation box. Here they were positioned sternally. Extubation was performed at the moment of recovery of the swallowing reflex. Then they were continuously observed until complete recovery and restoration of all reflexes. Postoperatively, they were administered the analgesic flunixin meglumine (Meflosyl, Fort Dodge Laboratories) 1.1 mg/kg i.v. for three days. The average anaesthesia time at replacement of both carotid arteries was 133 ± 28 min, the surgery time 100 ± 22 min.

At the extirpation of both prostheses and bilateral ligation of the carotid artery stumps (in two sheep implantation of a replacement prosthesis and stump ligation on the other side), anaesthesia lasted 97 ± 50 min and the surgery time was 75 ± 45 min.

Doppler ultrasound examination was performed to verify the patency of each implanted prosthesis.

Everyday care and observation were performed by professional staff (veterinarians, veterinary technicians and keepers). In case of contingent complication in an animal, a consultation of all the research participants would take place. In case of a problem the principal investigator would be informed and the problem would be solved. In case of no solution that would save the animal, a consultation with veterinarians and keepers would take place and the investigator would decide to sacrifice the animal.

In our experiment, tissue integration of the vascular prosthesis - implant - was tested in the arterial system of sheep in the early and late time periods.

Six prostheses were extirpated between days 95 and 104 after implantation and two on day 182 after implantation, and their macroscopic (Plate VIII, Fig. 2) and microscopic analysis (Plate IX Fig. 3) were made.

The histological description of the preferred vascular grafts implanted (Fig. 3) shows parts of the prosthesis with a well developed external fibrous coating, properly formed fibres of refractive heterogeneous material with focal minor chronic inflammatory cellularity and scattered giant multinuclear cells around the fibres. In the pores of the prosthesis, practically mature well-vascularized granulation tissue with infrequent chronic inflammatory cells, histiocytes and isolated neutrophils were found. Internal coating of the lumen (pseudointima) is formed by relatively cellular connective tissue of a maximum thickness of 0.7 mm with fibroblasts and infrequent lymphocytes. Circumferentially, the pseudointima is endothelialized.

This corresponds to the late phase of graft incorporation. Artery-to-prosthesis transition: fibrous tissue integration with good graft incorporation.

The animals were left alive. From previous experiments conducted by the first author on moufflons we can assume that the absence of the carotid bed is not a life-threatening condition for the sheep.

The study was approved by the Ethics Committee of the University of Veterinary and Pharmaceutical Sciences Brno.

Table 1. Number of days with the implanted prosthesis

	Sheep 1	Sheep 2	Sheep 3	Sheep 4	Sheep 5	Sheep 6	Sheep 7	Sheep 8
Prosthesis implantation	12. 5. 2007	12. 5. 2007	13. 5. 2007	13. 5. 2007	19. 5. 2007	19. 5. 2007	20. 5. 2007	20. 5. 2007
Prosthesis explantation	23. 8. 2007	1. 9. 2007	1. 9. 2007	23. 8. 2007	17. 11. 2007	17. 11. 2007	23. 8. 2007	1. 9. 2007
Days with the prosthesis	103	112	111	102	182	182	95	104
LCCA - Type of prosthesis	A	C	C	D	B	A	A	D
RCCA - Type of prosthesis	B	D	A	B	C	D	C	B
Patency of the left a. carotis	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Patency of the right a. carotis	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes

Results

The results of analyses and *in vivo* tests in the sheep were as follows:

In 6 sheep, the implantation period of a prosthesis was in the early time period (sheep No. 1 - 103 days, No. 2 - 112 days, No. 3 - 111 days, No. 4 - 102 days, No. 7 - 95 days, No. 8 - 104 days), i.e. the implantation period in these 6 sheep was 95 to 112 days, with the average of 104.5 days.

In 2 sheep the implantation period was classified as advanced (sheep No 5 - 182 days and sheep No 6 - also 182 days), thus the average period was 182 days.

The body mass of sheep was between 55 kg and 106 kg (mean = 83.125; median = 84.5; mode = 77).

Prostheses implantation time on both sides ranged between 56 and 80 min (mean = 64.875; median = 62.5; mode = 60 and 70).

Table 1 shows the operative protocol. It can be seen that in sheep number seven both prostheses were obliterated at the time of extirpation (weight = 106 kg; implantation time = 70 min; 15,000 heparin units). The left carotid artery of sheep number eight was obliterated as well (weight = 93 kg; implantation time = 65; 10,000 heparin units).

Detailed information on type of prostheses vs. patency is shown in Table 2.

All implanted prostheses of group B (with deposited DLC coatings of about 20 nm thickness with a high content of sp³ bonds (more diamond-like bonds)) were patent at the time of extraction, whereas in the other groups at least one was obliterated.

Discussion

Before clinical trials of the new type of vascular prosthesis in humans, assessment of rheological and immunological reactions to this prosthesis in a living animal with the *in vivo* method was necessary. The experiment could not be replaced by alternative *in vitro* methods. The number of animals was adjusted to the number of vascular prostheses delivered from the research and development centre of the Knitting Research Institute, Brno.

The experimental sample was very small and for that reason, the results obtained cannot be taken as representative but may be followed by further research on vascular prostheses for human use. Only healthy animals were included in the study.

From the results we have observed that two out of three vascular prostheses obliterated were located in sheep No. 7. This fact drew our attention. The only characteristic in this sheep that differed from the others was its body mass of 106 kg, being the heaviest of the group. Implantation time

Table 2. Operation protocol

	Ultrasound 9. 2007	Peroperatively (9. 2007)	Perioperatively (11. 2007)
Prosthesis A	3 patent 1 non-patent	2 patent 1 non-patent	3 patent 1 non-patent
Prosthesis B	4 patent 0 non-patent	3 patent 0 non-patent	4 patent 0 non-patent
Prosthesis C	3 patent 1 non-patent	2 patent 1 non-patent	3 patent 1 non-patent
Prosthesis D (Control group)	3 patent 1 non-patent	2 patent 1 non-patent	3 patent 1 non-patent

(70 min) was 14 min longer than the shortest implantation period (58 min) and 10 min shorter than the longest implantation time (80 min). The sheep was given 15,000 heparin units, the same amount as other sheep whose vascular prostheses were not obliterated. Despite this finding, this animal did not present any postoperative complications.

The other prosthesis obliterated was located in sheep No. 8 (body mass = 93 kg; implantation time = 65 min; 10,000 heparin units). This animal did not show any postoperative complications. We observed that the carbon-coated vascular prostheses exhibited reduced thrombogenicity and improved patency over non-carbon-coated grafts.

We believe that this prosthesis satisfies better the rheologic characteristics for healing. Vascular prosthesis group B proved to be the best. It is prosthesis with deposition of DLC coating of a thickness of 20 nm with a high content of sp³ bonds (more diamond-like ones). The implant lumen was constant: 7 mm. Evaluation was based on functionality, macroscopic and microscopic findings.

The findings are of a relative value and there is no guarantee that they will be fully applicable also in humans. Functionality of the vascular prosthesis can be deduced with great certainty but the opinion on quality and speed of tissue integration cannot be completely evaluated as cellular and extracellular activities differ in various animal species. Nevertheless, it can be stated that animal experiments will preliminarily exclude completely inconvenient and wrong technical procedures as well as unsuitable prostheses (implants).

Sheep were chosen as experimental animals because of the following similarities of their carotid arteries with humans:

- Behaviour of the arterial bed
- Carotid length and accessibility
- Endothelisation at anastomosis site

Another advantage is the fact that sheep require minimum care in the postoperative period and the follow-up is not too expensive (Krajiček et al 2007).

The length of the vascular prosthesis (implant) was 7 cm. Since the 1950s it has been known that the length of the implant must be at least 5.5–6 cm, otherwise the result does not correspond to the actual situation as the tissue integration takes place mainly as growth of cellular structures from the stump of the host's artery.

Based on the results, the experimental type B of prosthesis ARTECOR® appears to be the most successful among the tested prosthesis (at the end of the study all B-type remained patent). According to our results, it appears that carbon coated grafts exhibit reduced thrombogenicity and improved patency over non-carbon vascular grafts.

Further questions for research are why the prosthesis with more diamond-like bonds had better results than those with more graphitic bonds, and what influence the carbon width has on prosthesis patency.

The transfer of the results concerning vascular prostheses from experimental animals to humans requires further meticulous verification before its use in practice.

Experimentální ověření nového typu cévní protézy ARTECOR® pokryté uhlíkem na ovcích

Cílem studie bylo testování a ověření vlastností nových druhů cévních uhlíkem potažených protéz firemního označení ARTECOR® vyvinutých ve Výzkumném ústavu pletářském, a.s. Brno. Osmi zdravým ovčím plemene Merino, starým 2 až 3 roky byly implantovány čtyři typy (A, B, C s povrchem potaženém diamantu podobným uhlíku (DCL) a D jako kontrolní, bez DCL) cévních protéz. Místem implantace byla společná karotická tepna; délka implantace byla 7 cm. Všechny ovce dostaly profylakticky antibiotika v souladu s teorií tzv. "chráněného koagula". Ultrazvuková zkouška průchodnosti dle Dopplera byla uskutečněna před ukončením operace k ověření průchodnosti každé protézy. Během studie byla zvířata separována. U 6 ovcí byly protézy vytaženy přibližně za 100 dní, u 2 ovcí pak za 182 dní. Dle pooperační průchodnosti měly nejlepší výsledky protézy typu B. Implantáty měly stejný lumen a to 7 mm. U protéz typu B byl povrch potažen diamantu podobným uhlíkem ve vrstvě 20 nm.

Experimentální protézy ARTECOR® typu B se ukázaly jako nejlepší z testovaných protéz (všechny protézy typu B byly průchodné). Tyto protézy nejlépe vyhovovaly vlastnostem reologickým a byly dobře vhojené.

References

- Enzler MA, Zünd G, Schneider J. 1994: A new approach to *in vivo* testing of vascular grafts? *Vasa* **23**: 251-256
- Giardino R, Fini M, Rocca M, Nicoli Aldini NN, Martini L, Giavaresi G, Di Paola MD, Orienti L, Faggioli G, Gargiulo M 1995: *In vivo* experimental research in vascular surgery. Methodology and current Italian law. *Minerva Chir* **50**: 613-619
- Hauert R, Miller U 2003: An overview on tailored tribological and biological behavior of diamond-like carbon. *Diam Relat Mat* **12**: 171-177
- Krajiček M, Peregrin JH, Roček M, Šebesta P. 2007: Chirurgická a intervenční léčba cévních onemocnění. Grada Publishing, 436 p.
- Podlaha J, Kořístek V, Černý J, Riebel O, Šilhart Z, Fiala J, Gregor Z 1987: Experimental testing of new vascular prosthesis with small diameter. *Scr Med Brno* **60**: 235-242
- Podlaha J, Kořístek V, Riebel O, Gregor Z, Šilhart Z, Fiala J, Pospíšil L, Černý J 1987: The influence of a modified external surface of the artificial prosthesis with small diameter on better biocompatible properties. *Scr Med Brno* **60**: 471-474
- Ratto GB, Di Primio R, Romano P, Truini M, Badini A, Zaccheo D, Motta G 1988: New directions in the use of carbon as vascular graft material. *Vasc Endovasc Surg* **22**: 244-252
- Sawyer P 1987: Modern vascular grafts. Mc Graw Hill, New York, 326 p.
- Sharp W 1998: Carbon containing vascular graft and method of making same. US patent 5827327.
- Walter M, Erasmí H, Schmidt R 1991: A new biological vascular prosthesis. *Vasa Suppl* **33**: 90-91



Fig. 1. New type of vascular prosthesis

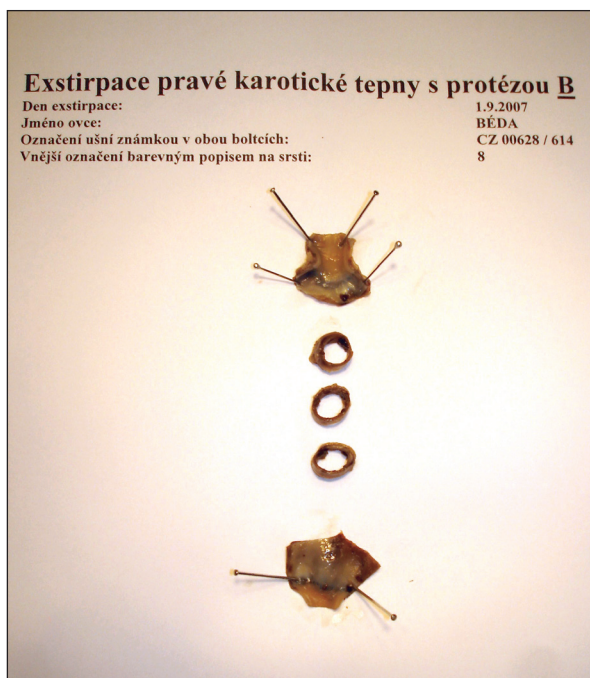


Fig. 2. Macroscopic analysis

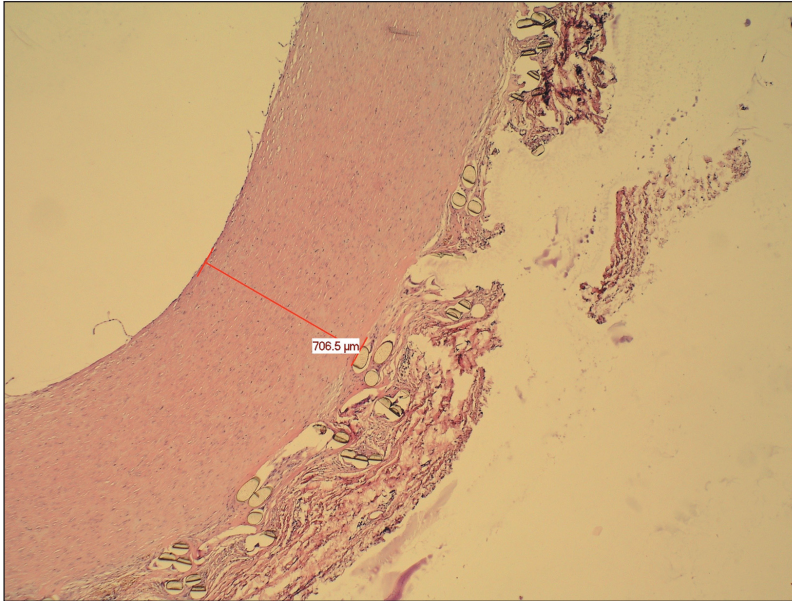


Fig. 3. Histology of the implanted preferred prosthesis B (HE, magnification $\times 40$)