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Bacterial synthesized cellulose — artificial blood vessels for microsurgery

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Abstract

Besides the most popular isolation of cellulose from plants the principal pathways of cellulose include the biosynthesis by different types of microorganisms, the enzymatic in vitro synthesis, and the chemosynthesis from glucose derivatives. The present paper describes the cellulose formation using *Acetobacter xylinum* and D-glucose as C-source. Kinetic investigations of the biosynthesis, methods of purification, and morphological investigations of the formed cellulose are reviewed and demonstrated by own results.

The properties of the bacterial cellulose are quite different from those of plant celluloses. That especially concerns the ultrafine network architecture, high hydrophilicity, and mouldability during formation.

BActerial SYnthesized Cellulose (BASYC®) was designed tubularly directly during the cultivation with the aim to develop biomaterials for medical application. These formed products were applicated as covers in experimental micronerve surgery and — most important — as artificial blood vessel interpositions with inner diameter of about 1 mm.

High mechanical strength in wet state, enormous water retention values, low roughness of the inner surface, and a complete 'vitalization' of BASYC® — microvessel-interpositions in rat experiments demonstrate the high potential of BASYC® as an artificial blood vessel in microsurgery.

Keywords: Acetobacter xylinum; Bacterial cellulose; BASYC®; Biosynthesis; Structure; Properties; Medical application; Microsurgery; Artificial blood vessel; Microvessel endoprosthesis; Micronerve cover; Microsurgical training

Contents

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1. Introduction

The topics of this contribution concern with biomaterials based on cellulose and formed by a biotechnological structure design.

The most important properties of cellulose like hydrophilicity, structure forming potential, chirality and biocompatibility as well as the molecular and supramolecular structure were continually modified by functionalization and regeneration processes.

In the present case the properties, structure, and shape of the biomaterial could be designed biotechnologically using *Acetobacter xylinum* (*A. xylinum*).

In detail, the introductory topic concerns the role of cellulose from the viewpoint of biomaterials and biotechnology. The subsequent chapter describes the formation of bacterial cellulose of *A. xylinum* as a very important type of cellulosic materials with properties different from plant celluloses. Furthermore the discussion is focused on biomaterials useful in medical fields. Sections 4 and 5 are dedicated to the synthesis and properties of novel types of cellulosic biomaterials (BASYC®: BActerial SYnthesized Cellulose) directly moulded during the cultivation process and its application in different purposes of experimental microsurgery.

Especially the development of BASYC® starting from the biotechnological formation of bacterial cellulose including shaping procedures and the microsurgical use as cellulosic endoprosthesis are the result of an effective cooperation of the experimental work of chemists, biologists and surgeons.

The investigations are incorporated in the world wide dramatic growth of biotechnology directed to pharmaceutical and medical products as well as of biomaterial design for human needs.

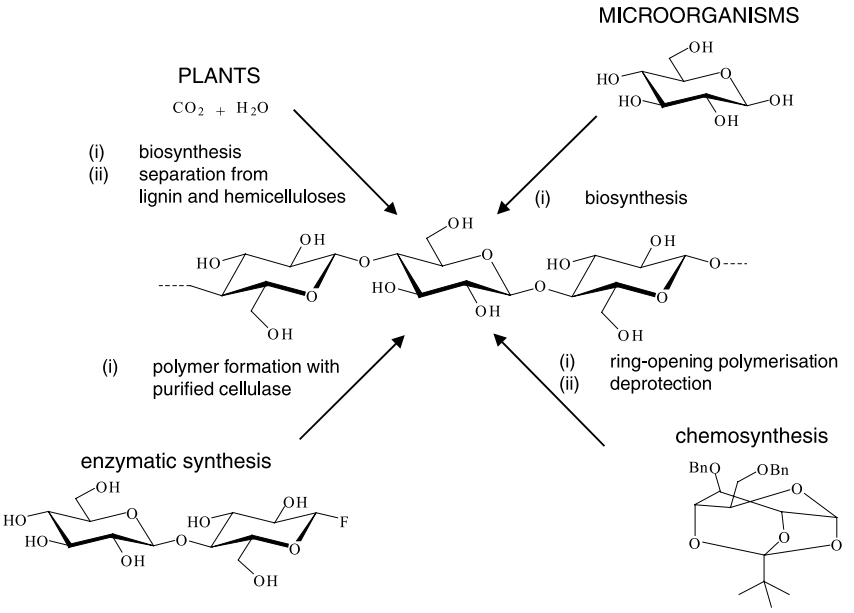

Fig. 1. Pathways to the cellulose [175]. Reproduced with permission from Kirshenbaum G, The Gordon and Breach Science Group. Polym News 24 (1999).

2. Bacterial cellulose

2.1. Principal pathways to cellulose

Up to now there are four different pathways to form the biopolymer cellulose. The first one is the most popular and industrial important isolation of cellulose from plants including separation processes to remove lignin and hemicelluloses [1,2]. The second way consists in the biosynthesis of cellulose by different types of microorganisms. Algae (Vallonia), fungi (Saprolegnia, *Dictystelium discoideum*), or bacteria (Acetobacter, Achromobacter, Aerobacter, Agrobacterium, Pseudomonas, Rhizobium, Sarcina, Alcaligenes, Zoogloea) are known from the literature [3,4]. But not all of these bacterial species are able to secrete the synthesized cellulose as fibers extracellularly.

From the scientific point of view the first enzymatic in vitro synthesis starting from cellobiosyl fluoride [5,6] and the first chemosynthesis from glucose by ring-opening polymerization of benzylated and pivaloylated derivatives [7] are of importance. These principle pathways are described schematically in Fig. 1.

2.2. Cellulose synthesis using A. xylinum

2.2.1. Fundamentals

Subject of the present paper is bacterial cellulose synthesized from *A. xylinum* lacking for photosynthetic capacity. Starting from the water-soluble, non-toxic monosaccharide D-glucose the non-pathogenic bacteria builds up pure cellulose extracellularly within some days. The cellulose synthesized by *A. xylinum* is identical to that made by plants in respect to molecular structure. However, the secreted

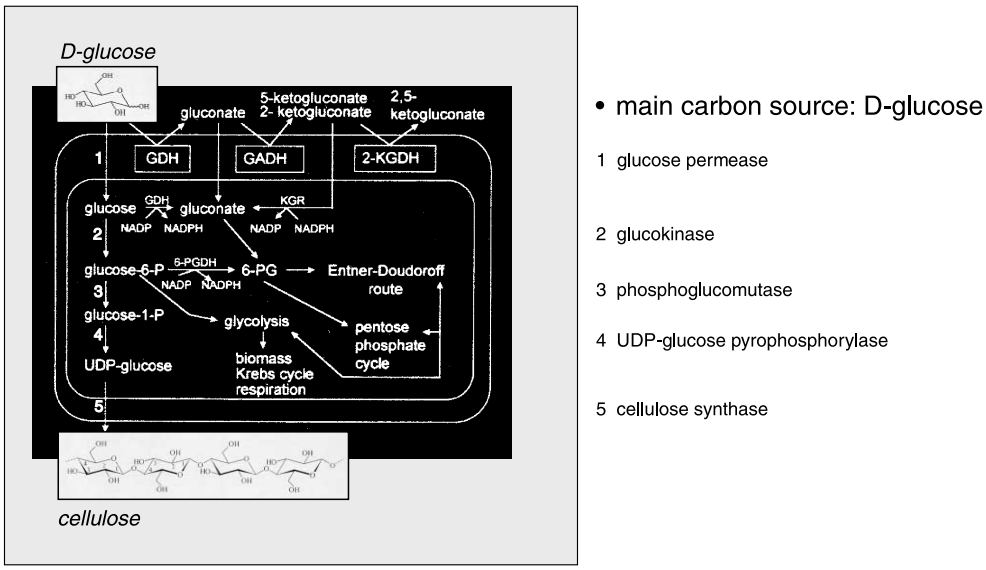

Fig. 2. Pathways of carbon metabolism in *A. xylinum* [10; modified]. Reproduced with permission from Wiley, J Chem Technol Biotechnol, vol. 67, 1996.

polysaccharide is free of lignin, pectin, and hemicellulose as well as other biogenic products, which are associated with plant cellulose. Additional, extracellularly synthesized microbial cellulose differs from plant cellulose with respect to its high crystallinity, high water absorption capacity, and mechanical strength in the wet state, ultra-fine network structure, mouldability in situ, and availability in an initial wet state [4,8–15].

For the first time, the bacterium *A. xylinum* was described in 1886 by Brown [16,17]. He identified a gelatinous mat formed in the course of vinegar fermentation on the surface of the broth as chemically equivalent to cell-wall cellulose. Microscopic observations disclosed bacteria distributed throughout the matrix. Up to now *A. xylinum* serves as a model organism to research cellulose biosynthesis, crystallization processes, and structural properties [9,14,18–46].

Under the classic cultivation conditions *A. xylinum* produces cellulose in form of pellicles at the air/liquid interface of the culture medium in static culture starting from D-glucose [8,47].

As known from the literature [4,10,48] the cellulose formation includes five fundamental enzyme mediated steps: the transformation of glucose to UDP-glucose via glucose-6-phosphate and glucose-1-phosphate and finally the addition of UDP-glucose to the end of a growing polymer chain by cellulose synthase (Fig. 2). Cellulose synthase (UDP-glucose: 1,4-β-D-glycosyltransferase; EC 2.4.1.12) is regarded as the essential enzyme in the synthesis process. It is subjected to a complicated regulation mechanism, which controls activation and inactivation of the enzyme [3].

A. xylinum forms the cellulose between the outer and the cytoplasma membrane. The cellulose-synthesizing complexes or terminal complexes (TC) are linearly arranged, and in association with pores at the surface of the bacterium. In the first step of cellulose formation glucan chain aggregates consisting of approximately 6–8 glucan chains are elongated from the complex. These subelementary fibrils are assembled in the second step to form microfibrils followed by their tight assembly to form a ribbon as the third step (Fig. 3). The matrix of the interwoven ribbons constitutes the bacterial cellulose

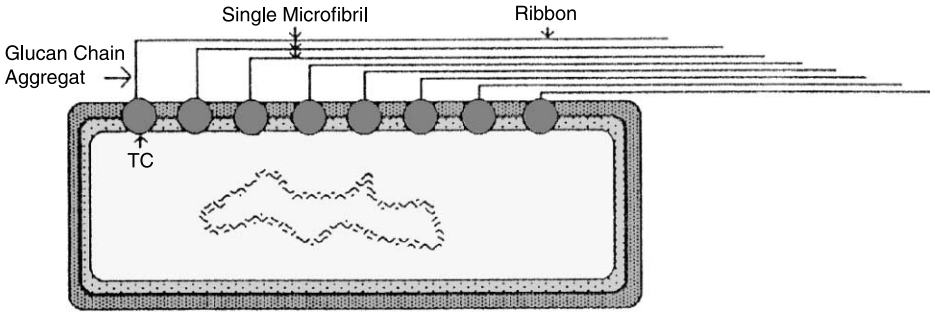

Fig. 3. Formation of bacterial cellulose [11]. Reproduced with permission from the author and the American Chemical Society, Harnessing biotechnology for the 21st century. Washington DC: American Chemical Society, 1992.

membrane or pellicle. Fig. 4 shows bacterial cellulose ribbon produced by one bacterial cell and Fig. 5 demonstrates that *A. xylinum* cells are distributed throughout the network of the cellulose ribbons [4,11,49,50].

Microorganisms of the genus Acetobacter are obligate aerobes and usually found on fruits, vegetables, in vinegar, fruit juices, and alcoholic beverages.

The synthesis mechanism helps the aerobic bacterial cells to arrive the oxygen-rich surface. Likewise, the pellicle protects the cells from the lethal effect of ultraviolet light, enhances colonization on fruits, retains moisture to prevent drying, and hold the bacteria in the aerobic environment [3,8–10,14,35,47,51,52].

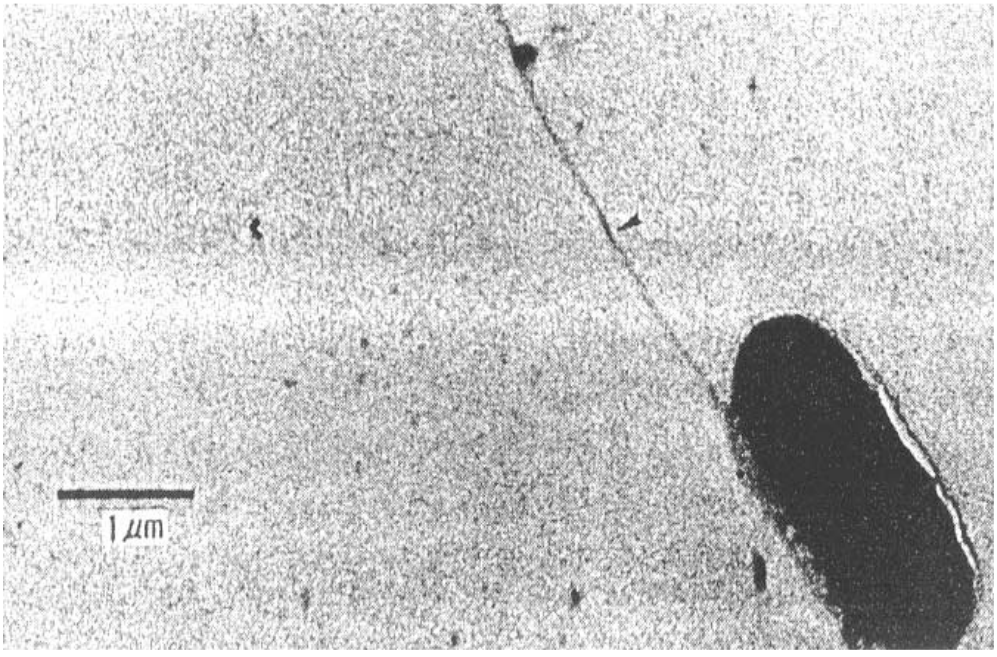

Fig. 4. Transmission electron micrograph of a bacterial cellulose ribbon produced by a bacterial cell [50]. Reproduced with permission from Hanser Verlag, Cellulosic polymers, blends and composites, 1994.

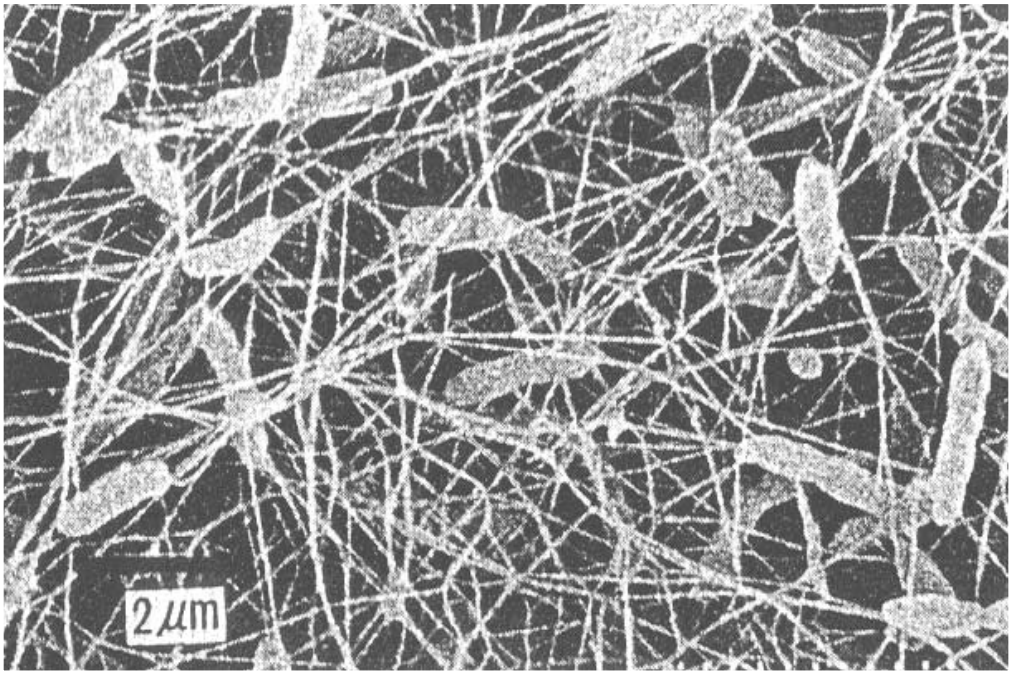

Fig. 5. Scanning electron micrograph of a bacterial cellulose network including the bacterial cells [50]. Reproduced with permission from Hanser Verlag, Cellulosic polymers, blends and composites, 1994.

Iguchi et al. assume that the bacteria construct a 'cage' and confine themselves in it to protect themselves from enemies and heavy-metal ions, whereas nutrients can be supplied easily by diffusion [53].

The special biosynthetic pathway of the *A. xylinum* animates scientists all over the world to modify the bacterial cellulose in vivo. In 1992 Ogawa and Tokura described the biochemical functionalization of a part of hydroxyl groups of the polyglucane by *N*-acetyl-D-glucosamine [54–56].

Besides the application of different glucose derivatives, possible C-sources for *A. xylinum* [57–60] were investigated.

The addition of water soluble polymeric substances like carboxymethylcellulose or hemicelluloses [25,26,32,49,61–66], chitosan [67], or fluorescent dyes [68] as well as enzymes like endoglucanase [69] influence the microstructure and the aggregation behavior. Kim et al. described the modification of bacterial cellulose using dextransucrase and alteransucrase [70]. By using carboxymethylcellulose or carboxymethylchitin as a component of the culture liquid partial carboxymethylated bacterial cellulose with adsorptive properties for metal ions and with good ion-exchange properties could be formed [71,72].

The typical features of bacterial cellulose have also inspired investigations on the reactivity and availability of the hydroxyl groups for chemical reactions [73–80].

Special fields of research were investigations on magnetization of bacterial cellulose by in situ synthesis of ferrites [81,82], on solution properties in metal-based solvents [83], or on behavior against NaOH solution [84,85].

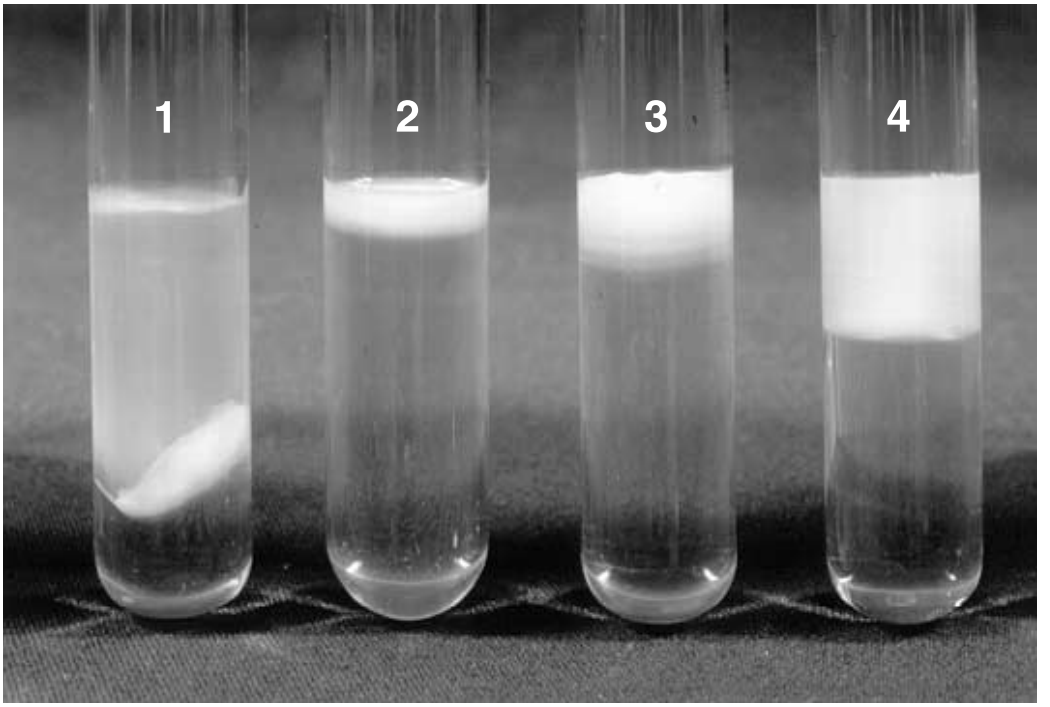

Fig. 6. *A. xylinum* strains cultivated on liquid Hestrin-Schramm-medium after 8 cultivation days: (1) ATCC 23769; (2) ATCC 10145; (3) ATCC 53582; (4) AX 5, not catalogued. Reproduced with permission from Kirshenbaum G, The Gordon and Breach Science Group. Polym News 24 (1999).

2.2.2. Kinetic investigations on substrate utilization and cellulose formation

At the beginning of our investigations we used different strains of *A. xylinum* (Fig. 6). From the tested strains the most effective cellulose formation to a very compact fleece has been observed in case of strain AX 5.

Whereas the extracellular formation of cellulose pellicles takes place during the cultivation of *A. xylinum* on a liquid medium (Hestrin–Schramm-medium [8]) the growth of bacterial colonies (Fig. 7) is observed during the cultivation on a solid medium (Hestrin–Schramm/agar-medium). The first existing smooth spheroid becomes after approximately 8 cultivation days a rough, crinkled colony. Later the colonies merge in a coherent sheet. The morphological development base on the external sheath of cellulose microfibrils surrounding the dividing cell mass [86].

The experimental investigations on biosynthesis, properties, and applications of bacterial cellulose were carried out by using the *A. xylinum* strain AX 5 (originally from the strain collection of the Institute of Biotechnology Leipzig; purified and optimized on the Institute of Organic Chemistry and Macromolecular Chemistry Jena). The bacterium was cultivated in Erlenmeyer flasks containing 16–20 ml Hestrin–Schramm-medium [8] in static culture at 28°C for 10–14 days as described by Hestrin [47].

Kinetic investigations in a discontinuous, static culture of *A. xylinum* AX 5 included studies of the utilization of the C-source D-glucose and the cellulose formation. After an adaptation period of 1–2 days the D-glucose concentration decreases rapidly (Fig. 8). Between the second and the fifth day 90% of glucose is consumed. The consumption of the C-source in time is in very good agreement with the

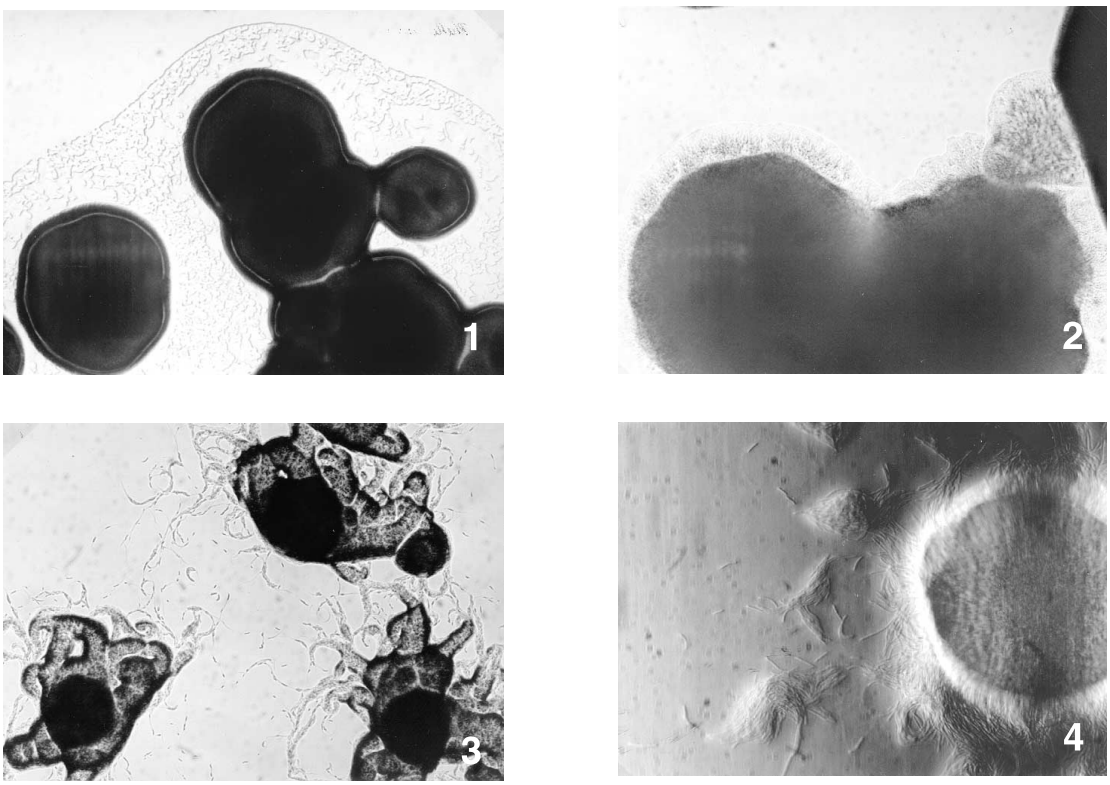

Fig. 7. Bacterial colonies of several *A. xylinum* strains on solid Hestrin-Schramm/agar-medium after 6 cultivation days: (1) ATCC 23769; (2) ATCC 10145; (3) ATCC 53582, original magnification 10×; (4) AX 5, not catalogued, original magnification 50×.

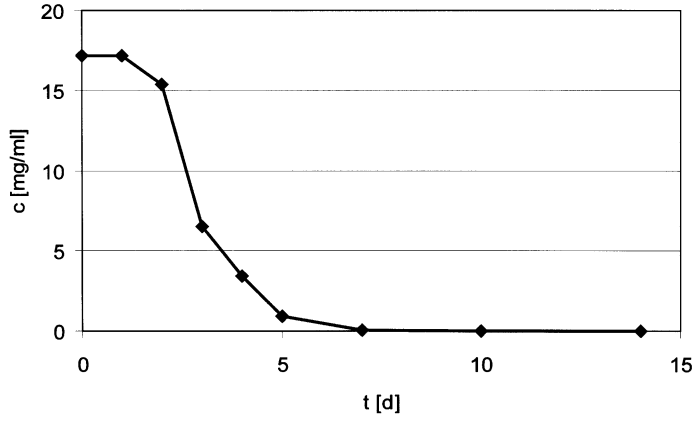

Fig. 8. Utilization of D-glucose by *A. xylinum* AX 5 (GOD-POD test).

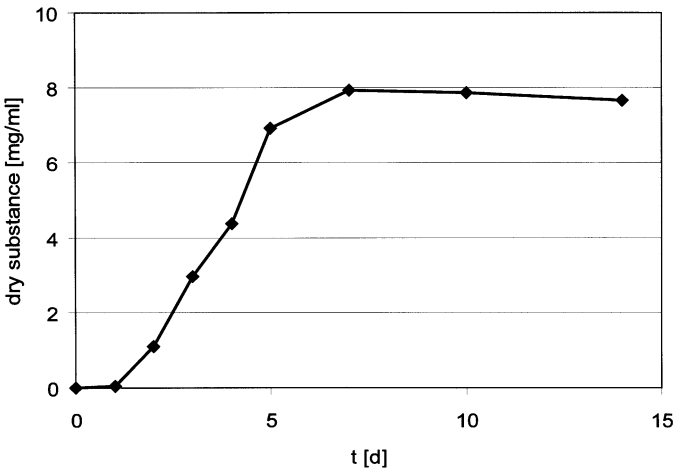

Fig. 9. Cellulose formation by *A. xylinum* AX 5.

amount of produced cellulose. In the range 8–10 days this processes are completed resulting in a high cellulose yield of 35–40% in relation to the applied glucose (Fig. 9). The utilization of the C-source was determined by using the GOD-POD test system (biochemical analysator YSI 2700 Select/Yellow Springs Instrument). The temporal cource of glucose consumption and cellulose production correlates with the experimental results of Fieldler et al. On the contrary to the authors we determined a complete utilization of the C-source [87].

D-glucose as the carbon source acts not only as an energy source but also as a cellulose precursor. The monosaccharide is also converted by membrane-bound Acetobacter dehydrogenase into (keto) gluconic acids. The conversion of glucose to (keto) gluconic acid is not beneficial for overall cellulose productivity. The sharp decrease in the medium-pH (final pH value of 3.5) probably limited not only cellulose formation, but also lowers the medium pH to suboptimal levels for cell viability and cellulose synthesis [3]. De Wulf et al. [10] have shown that mutants from *A. xylinum* LMG 1518 which are restricted in (keto) gluconate synthesis are able to produce cellulose more efficient. The reason why it was not possible to eliminate gluconate synthesis completely may be due to multiple availability of membrane-bound dehydrogenases. The authors assumed a vital function of the keto (gluconate) formation by *A. xylinum* because the dehydrogenases possibly play a part in a cell membrane-located electron transport chain. The effect of gluconic acid on the production of cellulose was also studied by Park et al. [88]. Investigations on wild-type cells and gluconate-negative mutants of the *A. xylinum* BRC5 showed that gluconic acid production has not only positive effects on cellulose biosynthesis but is also necessary for cellulose biosynthesis. The acid may affect cellulose production in the bacterium first by lowering the environmental pH and then possibly the internal pH, resulting in stabilization and/or activation of key enzyme(s) for cellulose biosynthesis.

By means of HPLC gluconic acid and 5-keto-gluconic acid were detectable in the culture broth of *A. xylinum* AX 5 during the cultivation. Whereas the appearance of gluconic acid could have been observed already from the second cultivation day in correlation with the started glucose utilization, 5-keto-gluconic acid as the oxidized secondary product could be determined only from the third day of cultivation. In the case of gluconic acid the concentration maximum was situated between the fifth

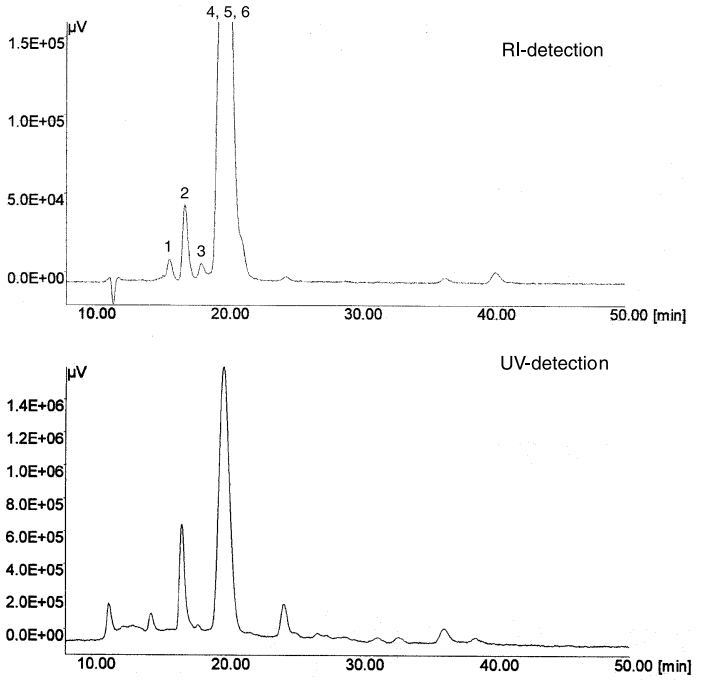

Fig. 10. HPLC elugramm of the culture medium inoculated with *A. xylinum* AX 5 after 4 cultivation days: (1) yeast extract; (2) citric acid; (3) 5-keto-gluconic acid; (4) D-glucose; (5) products of the sterilization process; (6) gluconic acid.

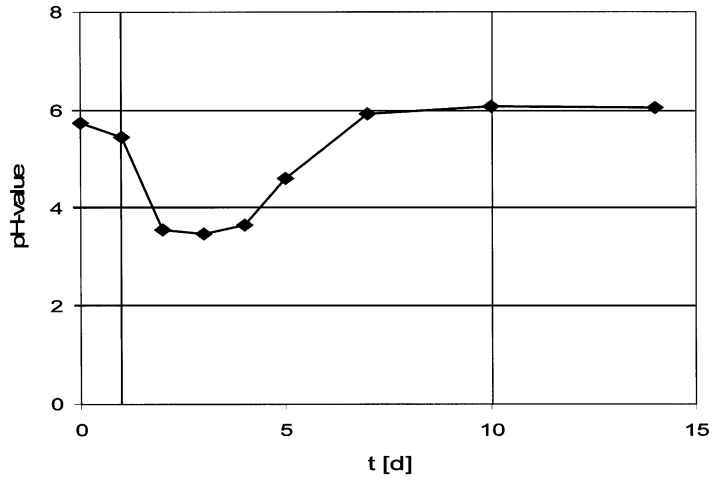

Fig. 11. Course of pH-value during the cultivation of *A. xylinum* AX 5 (| | period of appearance of gluconic acid and 5-keto-gluconic acid).

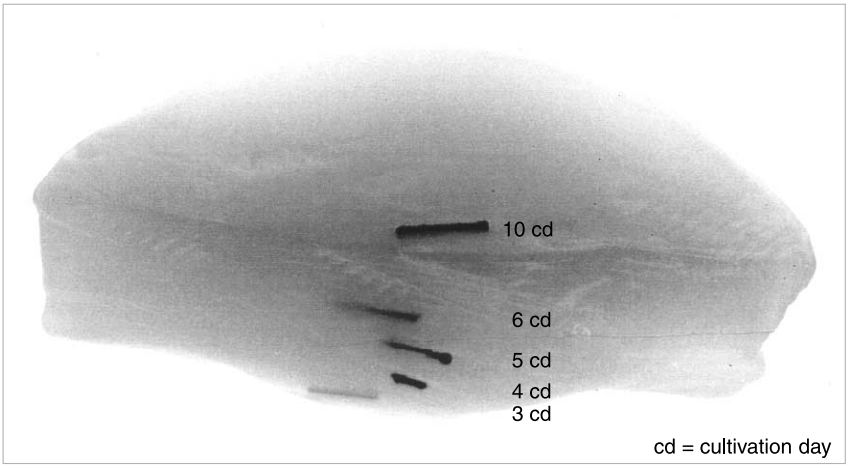

Fig. 12. Temporal development of the cellulose formation by marking experiments.

and the sixth day, in the case of 5-keto-gluconic acid the maximum was reached on the seventh cultivation day. At the end of the observation period, gluconic acid as well as 5-keto-gluconic acid were resumed. The components of the culture liquid were detected with a Biorad® system (column: 2 × Biorad aminex 87H, 300 × 7.8 mm^2; temperature: 65°C; mobile phase: 0.01N H_2SO_4, flow rate: 0.5 ml/min, RI and UV detector). Fig. 10 shows a typical HPLC elugramm of the culture broth after 4 cultivation days.

The appearance of the two acids gluconic acid and 5-keto-gluconic acid are responsible for the decrease of the pH-value of the culture medium during the first cultivation days (Fig. 11). In correlation to the literature [4,87,89] we observed pH-values between 4 and 6.

As mentioned above the aerobic bacterium *A. xylinum* synthesizes cellulose at the air/liquid interface of the culture medium in static culture. After an initial stage the formation of the cellulose takes place on the upper site of the cellulose layer. As long as the system is kept unshaken, the disc-shaped product is suspended and slides steadily downwards as it thickens so that components of the culture liquid have to diffuse through the synthesized fleece [90]. In detail, the mechanism of cellulose growth is assumed as follows. In the initial stage, the bacteria increase their population by consumption of oxygen initially dissolved in the medium. During this time, they synthesize a certain amount of cellulose in the liquid phase. Only bacteria, which exist in the vicinity of the surface and associate with oxygen, can maintain their activity and produce cellulose. Those bacteria below the surface of the pellicle are dormant. They can be reactivated and used as the inoculum for new culture operations [13,51,90]. In accordance with the method of Borzani and de Souza [91] we carefully added noticeable coloured pieces of twines on the surface of the produced supernatant cellulosic film in dependence on the cultivation time (Fig. 12). After one cultivation day the gelatinous cellulosic product did not retain the added twine. On the second cultivation day the twine was already integrated in the gelatinous upper part of the pellicle. Between the third and the sixth day of cultivation the twines were covered with a pronounced cellulose layer. And from the 10th day no integration of the added twine was observed. The cellulose formation process had finished.

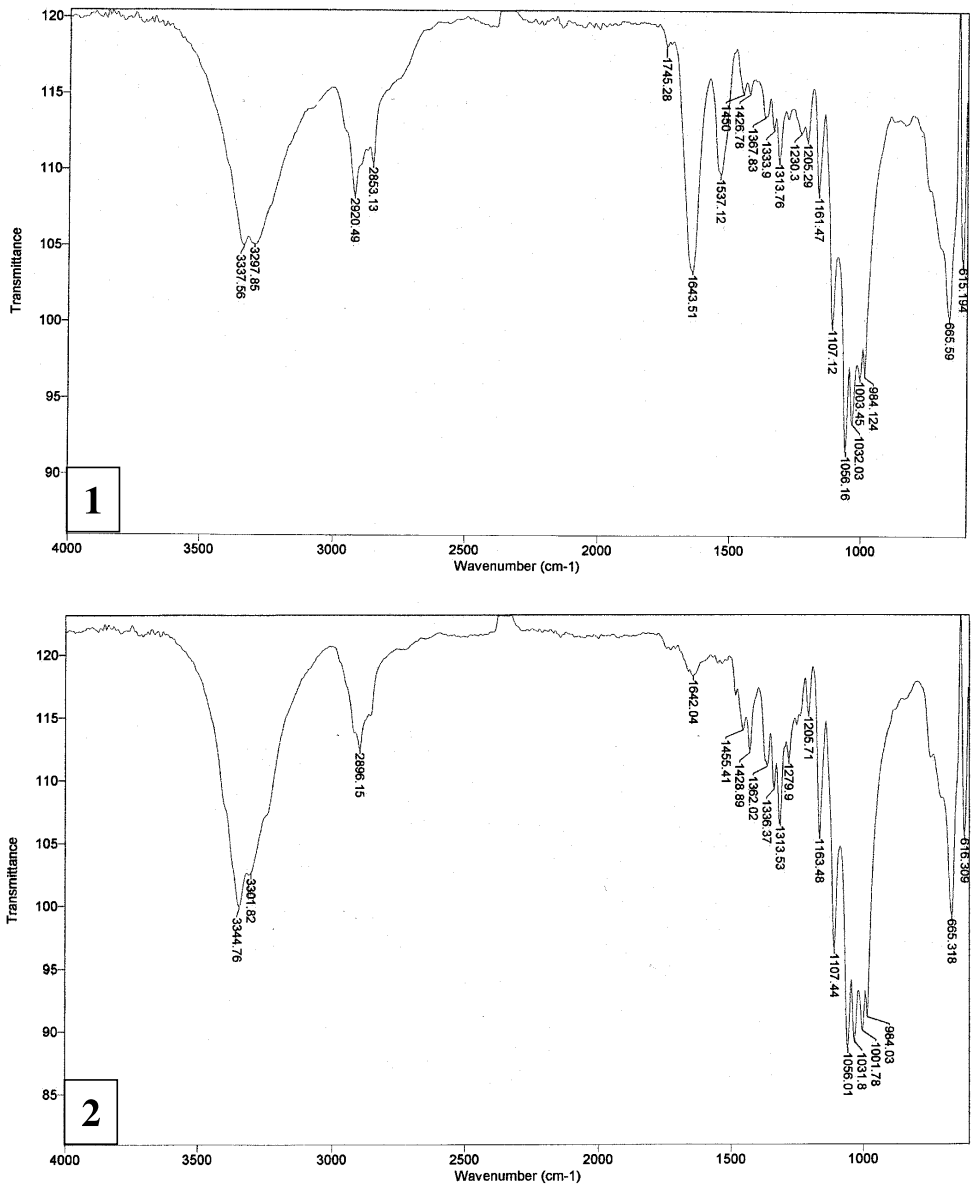

Fig. 13. Comparison of FT-IR spectra of bacterial cellulose: (1) washed with water; (2) purified with 0.1N NaOH (air drying, ATR-technique).

2.2.3. Purification of cellulose

As a suitable purification method for the bacterial cellulose pellicle we used the treatment with boiled, diluted sodium hydroxide to eliminate *A. xylinum* cells as well as components of the culture liquid, which are integrated within the cellulose network [92,93]. Results of the elementary analysis show a drastic decrease of amino compounds by the described purification method

Table 1
Results of the elementary analysis of bacterial cellulose pellicles after washing with water and after treatment with diluted NaOH solution in comparison with the calculated values of cellulose

Purification method	C (%)	H (%)	N (%)
H_2O	44.05	6.28	1.49
0.1N NaOH	43.77	6.14	0.13
Cellulose (calculated values)	42.39	6.39	–

(Table 1). The FT-IR spectra recording on a Digilab FTS 25 (BioRad) correlate with the values obtained from the elementary analysis. After NaOH treatment of the cellulose pellicles a drastic reduction of the amid I ($\nu(\text{strong})_{C=O}$: 1643 cm^{-1}) and the amid II signal (δ_{NH}: 1537 cm^{-1}) was determined (Fig. 13).

2.2.4. Morphological investigations

Investigations of the texture of bacterial cellulose have shown that a surface layer built on the interface between the culture broth and the air delimitates the pellicle surface clearly. A gelatinous lower layer is adjacent to the broad middle layer (Fig. 14).

By REM investigations of bacterial cellulose pellicles washed only with water, we could determine a surface layer of 6 μm thickness. The layer is characterized by a compact cellulose network structure and an intensified presence of bacterial cells (Fig. 15).

Analytical investigations corroborated the electronmicroscopic results. The percentage of nitrogen in the surface layer was significantly higher than in the middle layer (Table 2).

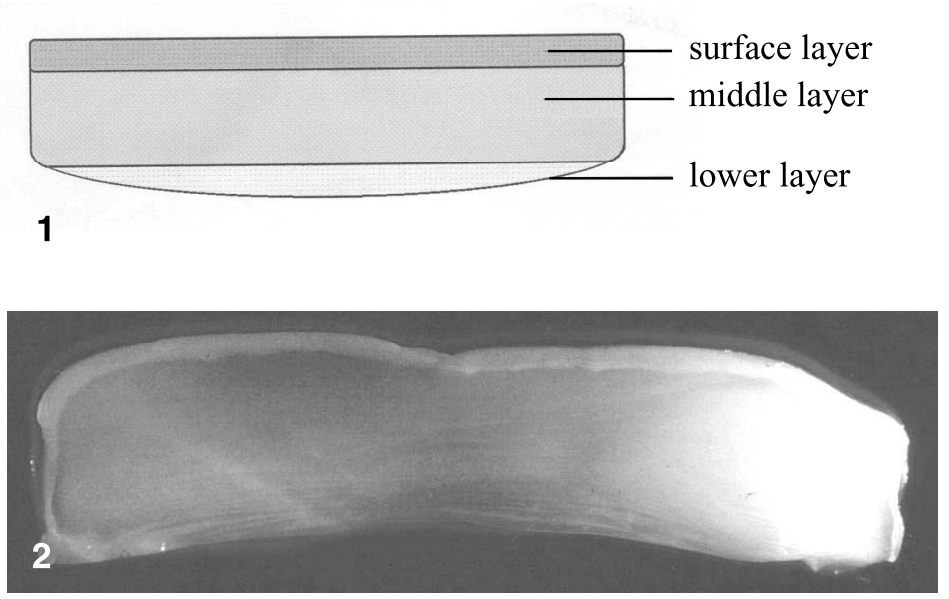

Fig. 14. Representation of cellulose layers inside the pellicle: (1) schematic representation; (2) cross-section of a purified bacterial cellulose pellicle.

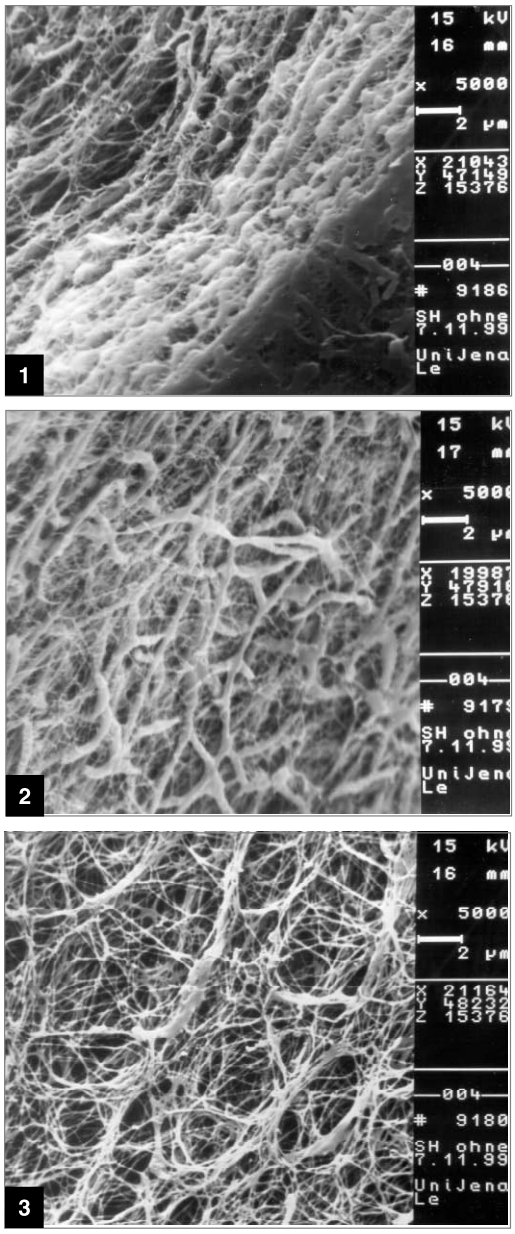

Fig. 15. Scanning electron micrographs (sample preparation: freeze drying) of surface and middle layer in the cross-section of a cellulose pellicle washed with water: (1) frontier area between surface and middle layer; (2) section of the surface layer; (3) section of the middle layer.

After treatment of the cellulose pellicle with 0.1N NaOH, we could determine a reduction of impurities and an alteration in the texture. The surface layer came up to a thickness of approximately 100 μm. A marked network structure was no more observed. In comparison with the untreated sample the cellulose fibers of the middle layer were more concentrated (Fig. 16).

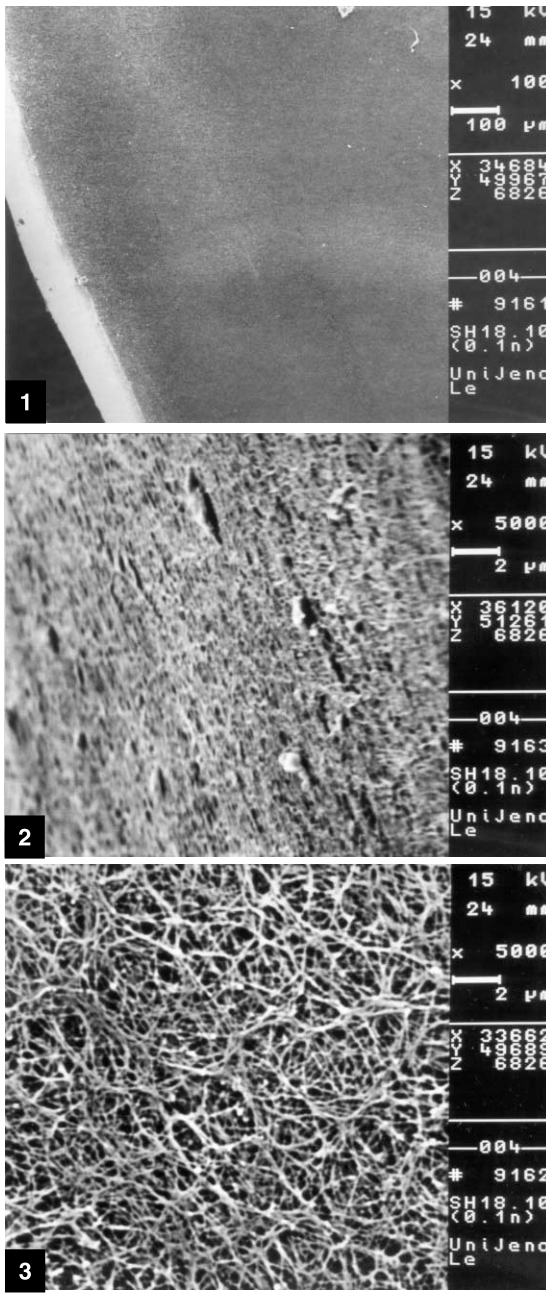

Fig. 16. Scanning electron micrographs (sample preparation: freeze drying) of surface and middle layers in the cross-section of a cellulose pellicle purified with 0.1N NaOH: (1) frontier area between surface and middle layer; (2) section of the surface layer; (3) section of the middle layer.

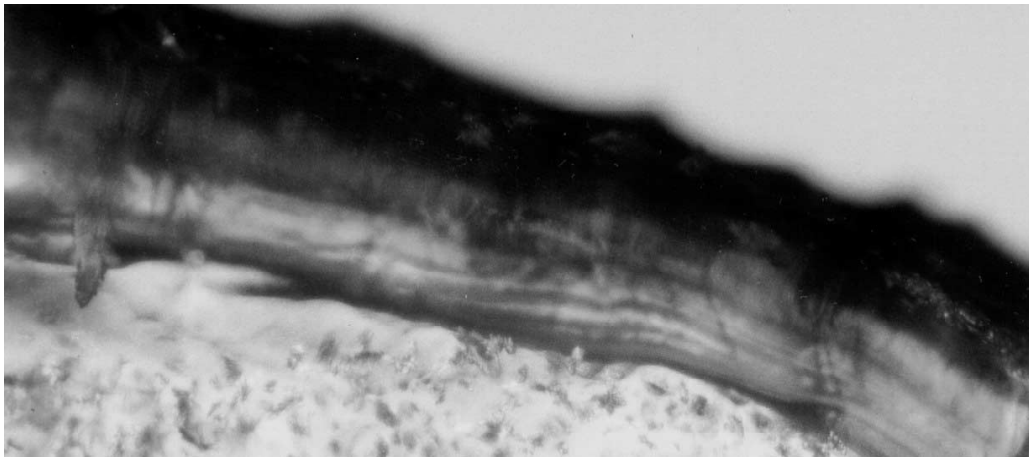

Fig. 17. Micrograph of the frontier area between surface and middle layer within a cellulose pellicle loaded with $CoSO_4$ (original magnification 3.2×).

Diffusion of aqueous $CoSO_4$-solution and subsequent crystallization of the salt within the purified pellicle by solvent transfer have shown that only in the middle and the under region of the cellulosic product sulphate crystals could be formed. Additionally, investigations on microtome cuts revealed that it was not able to integrate OsO_4 in the upper region of the fleece (Fig. 17).

2.3. Specific properties of bacterial cellulose

2.3.1. Ultrafine network structure

Bacterial cellulose is characterized by an ultrafine network structure. The comparison of the dimensions of bacterial cellulose fibers with natural and synthetic materials shows this advantage (Fig. 18). In Fig. 19 the comparison of bacterial cellulose and a thrombocyte as one of the smallest blood components with a diameter of about 3 μm gives an idea about the size of the hollow spaces within the cellulose network.

2.3.2. Hydrophilicity

During the cultivation *A. xylinum* AX 5 produces cellulose as a high swollen fiber network. The

Table 2
Results of the elementary analysis of bacterial cellulose layers

Sample/purification method	C (%)	H (%)	N (%)	S (%)
Surface layer/treatment with water	63.45	9.86	2.85	0.18
Middle layer/treatment with water	44.71	6.68	1.47	–
Surface layer/treatment with 0.1N NaOH	43.89	6.30	0.23	–
Middle layer treatment with 0.1N NaOH	44.16	6.56	0.25	–

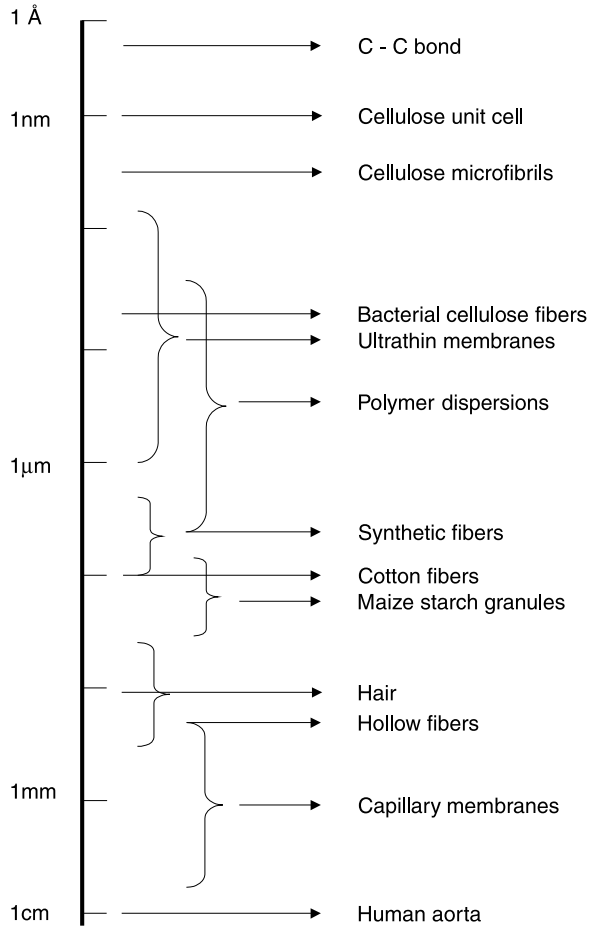

Fig. 18. Range of structure design of polysaccharides in comparison with synthetic and living materials.

hydrophilicity of the cellulose pellicle is explained by the presence of pore structures and 'tunnels' within the wet pellicle and depends on the extensive interior surface area of the interstitial spaces of the never dried matrix [12].

The demonstration of the water retention of never dried bacterial cellulose leads to values in the range of 1000%. It is important to note that the water retention values (WRV) seem to depend on the volume of the culture liquid. As shown in Fig. 20 the water retention values of typical plant cellulose like cotton linters are amounted under comparable conditions to values of about 60%. In comparison with the literature it confirms that the solvent retention values (SRV) of never dried bacterial cellulose were significantly higher than that for plant cellulose even under activation conditions (Table 3).

After air-drying the bacterial cellulose up to 100°C and reswelling with water at 30°C for more than 2 h, the WRVs were drastically decreased and comparable with those of plant cellulose. Freeze-drying was proved to be a more considerate drying method relating to the maintenance of pore structures.

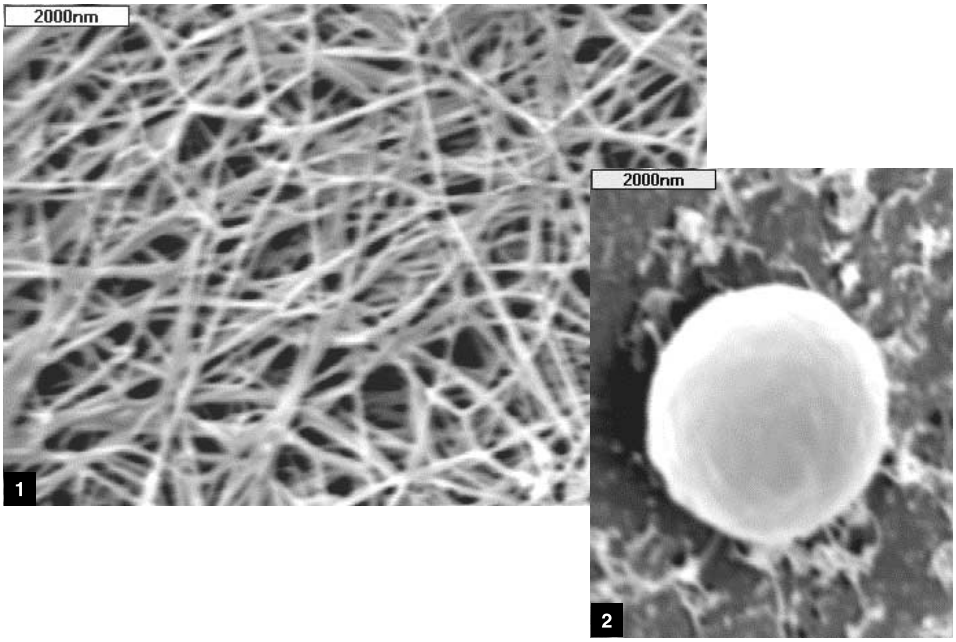

Fig. 19. Demonstration of the size of the hollow spaces within the cellulose network (1) in comparison with a thrombocyte (2).

The weakly included water of the never dried bacterial cellulose was removable by organic solvents, e.g. methanol, acetone, hexane. The determined solvent retention values did not significantly deviate from the water retention value [73].

The WRV was determined according to the method described by Jayme and Rothamel [94].

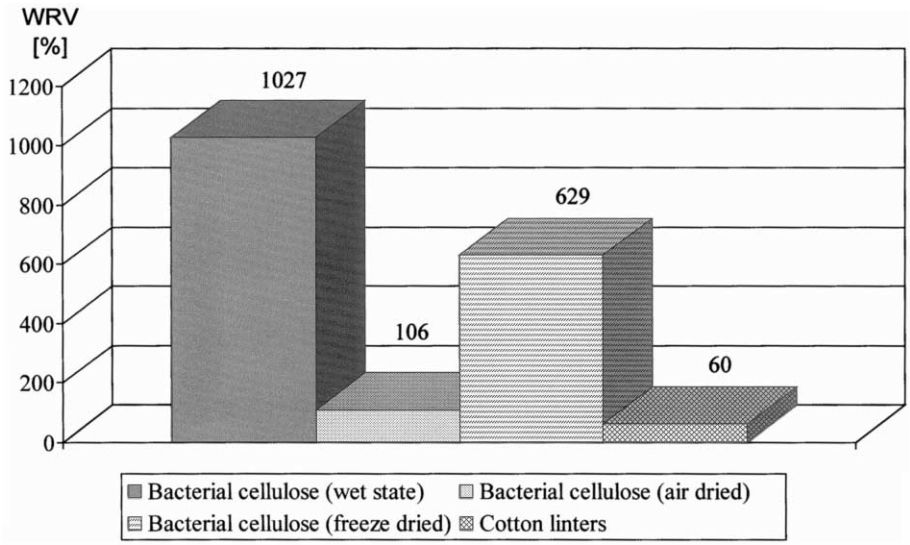

Fig. 20. Water retention values (WRV) of bacterial cellulose and plant cellulose.

Table 3
SRV of different kinds of cellulose

Kind of cellulose	SRV (%)			Literature
	Ethanolamine	Water	Methanol	
Spruce pulp	163	63	33	[190]
Viscose never dried	280	95	46	[190]
Viscose dried	256	86	25	[190]
Cotton	106	51	26	[191,192]
Cotton linters treated with CH_2NH_2/ $H_2N(CH_2)OH$	n.s	118	n.s	[193]

2.3.3. Transparency

Fig. 21 shows a photograph of a purified and air-dried bacterial cellulose pellicle taken on a printed sheet of paper to demonstrate the transparency of the network structure.

3. Bacterial cellulose for medical application

Due to its high purity and unusual physico-chemical properties bacterial cellulose offers a wide range of special applications, e.g. as a food matrix (nata de coco), as dietary fiber, as an acoustic or filter

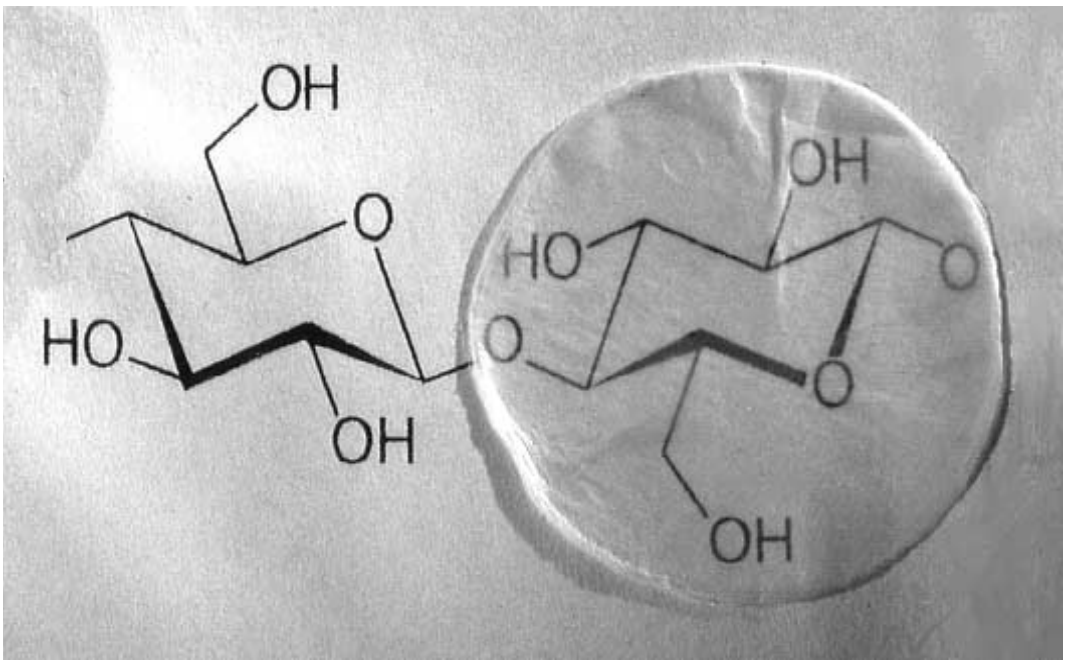

Fig. 21. Demonstration of the transparency of a purified and air-dried bacterial cellulose pellicle.

membrane, as ultra-strength paper and as a reticulated fine fiber network with coating, binding, thickening and suspending characteristics [3,4,35,50,51,95–99].

Up to now several applications of bacterial cellulose in human and veterinary medicine are known. The high mechanical strength in the wet state, substantial permeability for liquids and gases, and low irritation of skin indicated that the gelatinous membrane of bacterial cellulose was usable as an artificial skin for temporary covering of wounds.

Biofill® and Gengiflex® are products of bacterial cellulose with wide applications in surgery and dental implants and realities in the human health-care sector [4]. Cases of second and third degree burns, ulcers and others could be treated successfully with Biofill® as temporary substitute for human skin [100]. The authors documented the following advantages for Biofill in more than 300 treatments: immediate pain relief, close adhesion to the wound bed, diminished postsurgery discomfort, reduced infection rate, easiness of wound inspection (transparency), faster healing, improved exudates retention, spontaneous detachment following reepithelization, and reduced treatment time and costs. Only one disadvantage was mentioned: limited elasticity in areas of great mobility.

Gengiflex® was developed to recover periodontal tissues. Novaes and Novaes described a complete restoration of an osseus defect around and IMZ implant in association with a Gengiflex® therapy [101,102].

Further results about the application of Gengiflex® and Biofill® were published by different authors [103–112].

Schmauder et al. described the application of bacterial cellulose (Cellumed) in veterinary medicine to treat recent, large surface wounds on horses [113].

In experiments with dogs biosynthetic cellulose was also successfully applied to substitute the dura mater in the brain [114].

Through the use of gas permeable molds, cellulosic products of virtually any shape could be produced. White and Brown have demonstrated that a molded, seamless product in the shape of a glove can be formed in situ by Acetobacter [12].

The moldability of bacterial cellulose during the cultivation process, e.g. to a hollow cellulose tube applicable as substitution material for blood vessels or other internal organs was also described in different patents [115–120].

On the one hand the patented biotechnological methods have not described a microsurgical application and on the other hand the described methods seem to be bound up with disadvantages for the quality of the inner surface of the synthesized tubes.

4. Microvessel endoprostheses from BASYC®

4.1. Formation

The development of bacterial cellulose biomaterial useful as microvessel endoprostheses requires ‘right connections’. That means an effective cooperation between natural scientists and physicians as well as a good interaction and fit between the artificial soft material and the target organs of the body. From this point of view the novel styled biomaterial BASYC® was designed and characterized together with chemists, biologists, and surgeons.

A very important advantage of the microbial cellulose is their moldability in situ [12]. As described

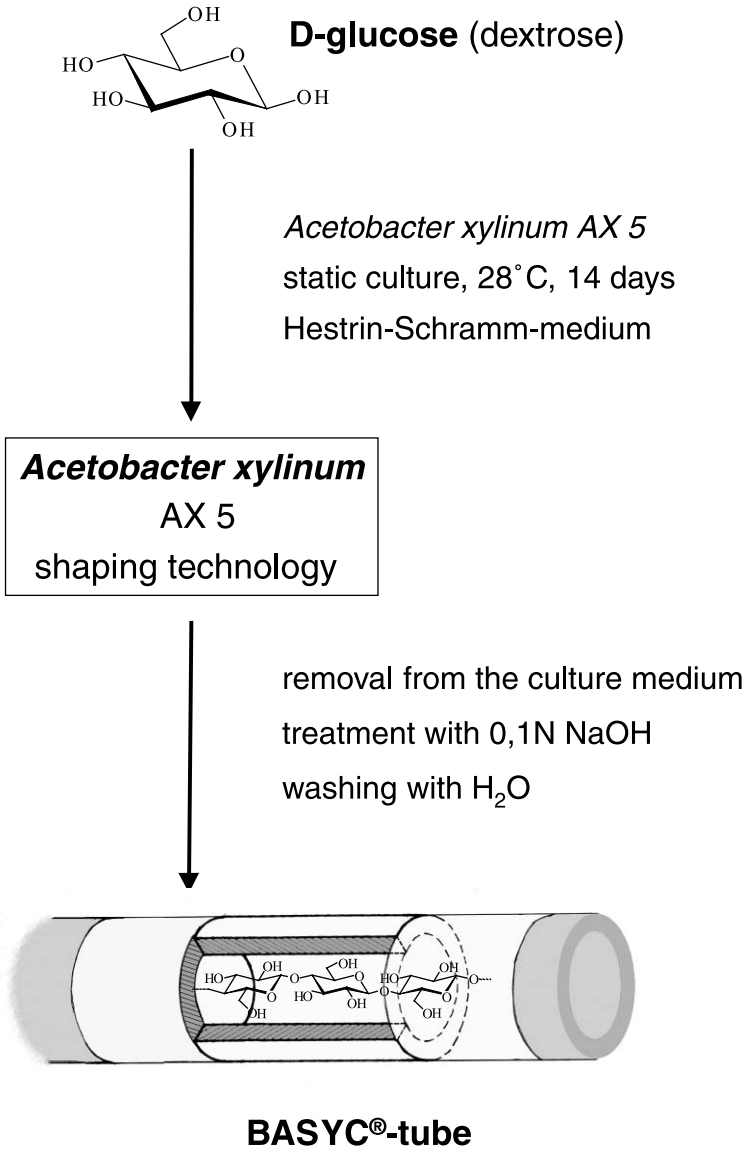

Fig. 22. Scheme of BASYC®-tube formation by *A. xylinum* AX 5.

schematically in Fig. 22 the formation of BASYC® proceeds in a static culture using the typical Schramm–Hestrin-medium [8] including D-glucose (dextrose) as the starting C-source. In other words, the direct 'growing' of cellulosic microvessels takes place during the cultivation of *A. xylinum*.

Using a patented matrix technique *A. xylinum* is able to build up the previously described cellulosic network as very regularly formed tubes of different length, wall thickness and inner diameter (Fig. 23). The marked BASYC®-tube has an inner diameter of 1 mm, length of about 5 mm, and wall thickness of 0.7 mm. These parameters are sufficient for experimental microsurgical requirements.

After removal of the tubes from the culture medium a simple purification method including washing

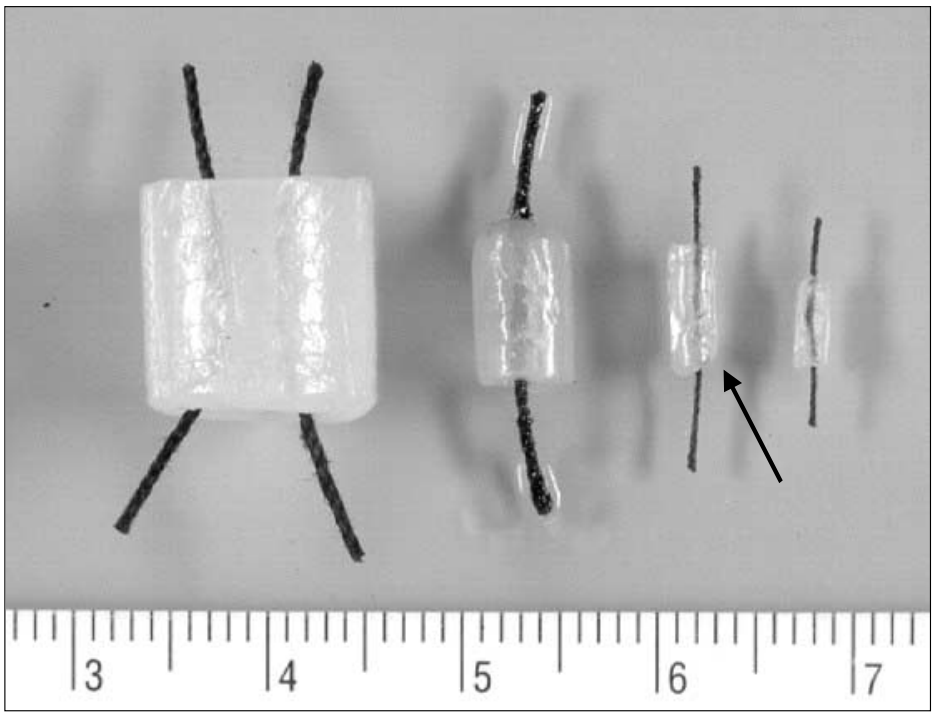

Fig. 23. BASYC®-tubes with different inside diameter, different wall thickness and different length. (→ BASYC®-tube sufficient for experimental microsurgical applications).

with water is necessary (see Section 2.2.3). Exchanging the swelling agent water with physiological salt solution the BASYC®-tubes can be stored about 6 weeks under cooling (4°C).

4.2. Properties

As discussed in Section 2.3, the properties of bacterial cellulose are quite different from the well-known properties of plant celluloses. Additional to hydrophilicity and ultra-fine network structure specific properties important for application as artificial microvessels have been characterized.

4.2.1. Roughness of the inner surface

Using interference microscopic investigations the inner surface quality of the designed, never-dried biomaterial could be demonstrated.

The measuring technique — interferometry in reflected light — is a contact- and destruction-free method. The measurements are based on the principle of the total picture shearing. Special software calculates surface parameters like roughness and allows graphical representations of the results. In detail, the investigations of the inner surface of the wet BASYC®-tubes were carried out on a JENA-MAP-apparatus system in combination with the interference microscope JENAVERT interphako v. (Carl Zeiss Jena). The conditions of the measurement are dry objective 25×0.50; vertical illumination, 100 W xenon lamp.

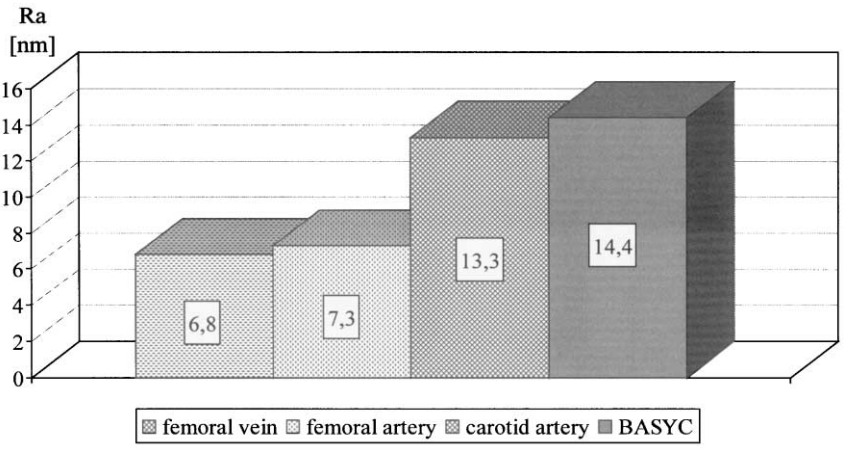

Fig. 24. Average values of roughness (R) of BASYC®-tubes and blood vessels of the rat. (R_a-arithmetical average of roughness = average deviation of the most adapted surface area.)

Fig. 24 summarizes average values of roughness of BASYC®-tubes and selected veins and arteries of the rat in their never dried state. Although the living and the artificial material show drastic structural differences (Fig. 25), the roughness of the inner surface of the BASYC®-tubes is comparable with that of the blood vessels and ranges between 7 and 14 nm.

4.2.2. Mechanical strength

A sufficient mechanical strength of the BASYC®-tubes is one of the essential properties for their use in microsurgery. The material must resist both mechanical strain during microsurgical preparation and anastomosing and blood pressure of the living body. The native bacterial cellulose has mechanical properties, including shape retention and tear resistance, which are superior to many synthetic materials [12].

In comparison with organic sheets, like polypropylene, polyethylene-terephthalate or cellophane the bacterial cellulose processed into a film or sheet show remarkable mechanical strength. This result was explained by the high crystallinity of bacterial cellulose, the high planar orientation of the ribbons pressed into a sheet, the ultrafine structure and the complex network of the ribbons [50]. Because of the high mechanical stability and low density, sheets of bacterial cellulose are useful for industrial purposes, e.g. in acoustic diaphragms. It is also known from the literature that paper made from bacterial cellulose is much stronger than papers made from ordinary pulps and that disintegrated bacterial cellulose seems to be useful for reinforcing various types of papers [13,121].

After preparation, watering and purification of the never-dried BASYC®-tubes in the manner described above (Fig. 22) the molded cellulose showed a stable form and consistency under normal handling and by application of microsurgical techniques (compare with Section 5.2).

In order to obtain more information about the mechanical stability of BASYC®-tubes first tension tests have been realized. For comparison several vessels of the rat were also examined. Therefore the BASYC®-tubes and the vessels were transversally sliced into segments of 2 mm length.

The never dried, cylindrical pieces are placed on a special developed supporting facility of a

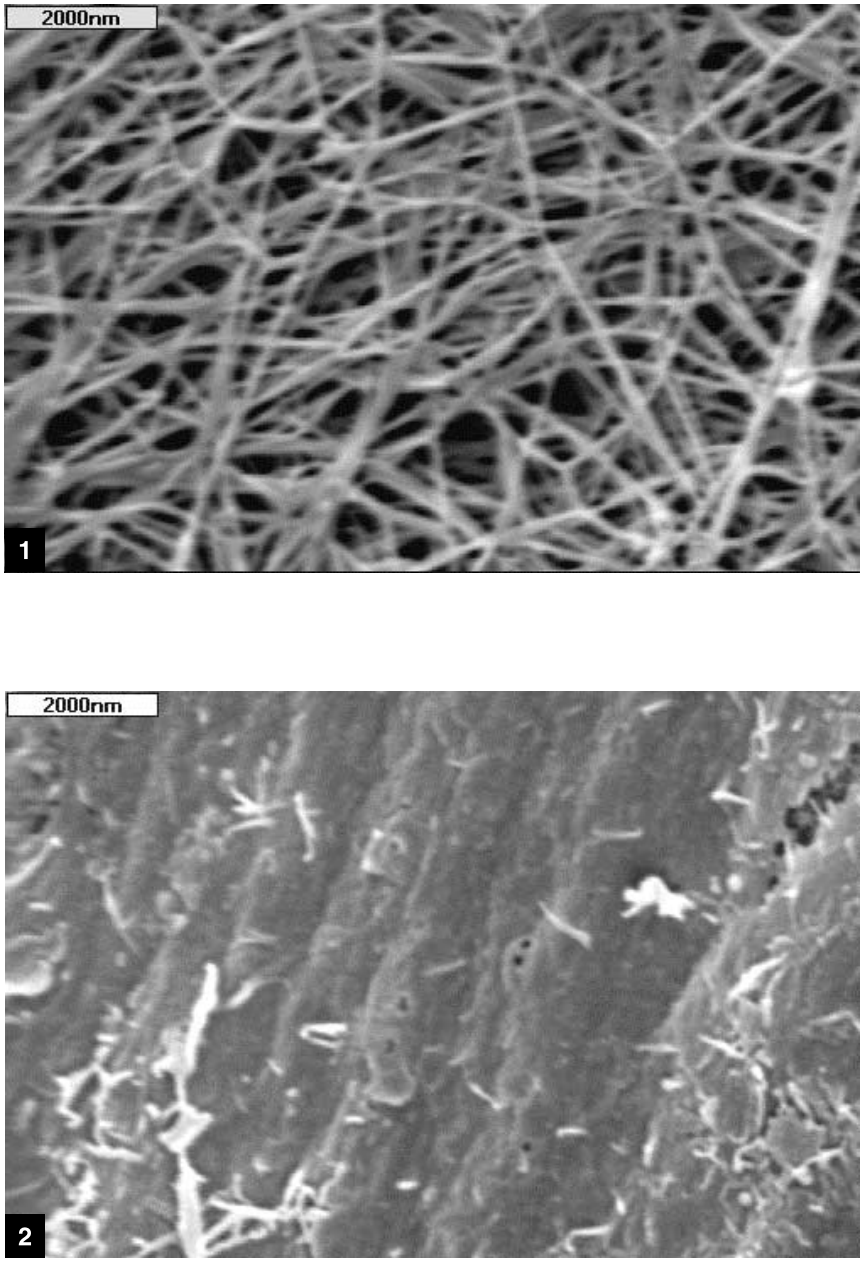

Fig. 25. Scanning electron micrographs (sample preparation: critical point drying) of inner surface areas: (1) BASYC®-tube; (2) carotid artery of the rat.

commercial tension test apparatus — the ZWICK-universal tester UPM 1445 including a special fixation device. A tensile force was applied with a speed of 1 mm/min. The experiment was finished when the tensile force was declined on 50% of the maximum amount. Fig. 26 shows schematically the principle of the test and the average values of the maximal tensile force (F_{max}) were shown in

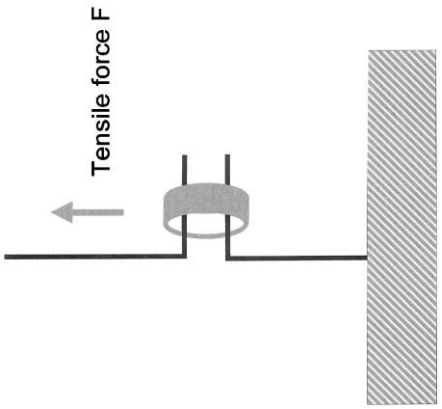

Fig. 26. Schematic representation of the tension test.

Fig. 27. It is reflected in the values that the load capacity of BASYC® and those of tested blood vessels are comparable and ranged in the mean value of about 800 mN.

An important aspect for the discussion of data obtained in the tension test is the definition of the wall thickness of the BASYC®-tubes and the different vessels. The wall thickness of wet BASYC®-tubes is determined by dimensions of the matrix (0.7 mm). However, measuring of wall parameters of native preparations of blood vessels proved quite inexact. So we used for the comparison of the wall thickness values, determined from histologic preparations (Fig. 28). The problem of this method consists in the shrinkage of the materials. Another difficulty is the variation of vessel parameters in dependency on size, age, weight and sex of the animals. With the method of tension measurement, it is possible to determine a value, suitable for the comparison of the probes among each other — the maximal tension force (F_{max}). But an accurately defined quantitative evaluation

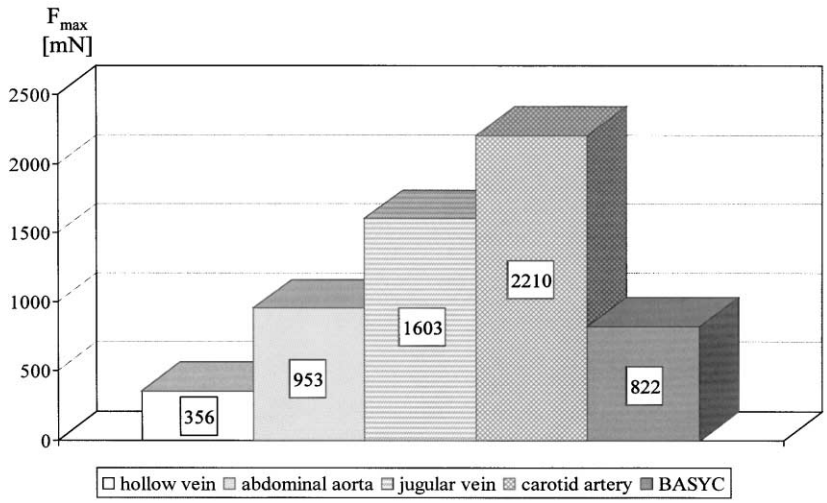

Fig. 27. Average values of maximal tensile force (F_{max}) of several blood vessels of the rat and BASYC®-tubes.

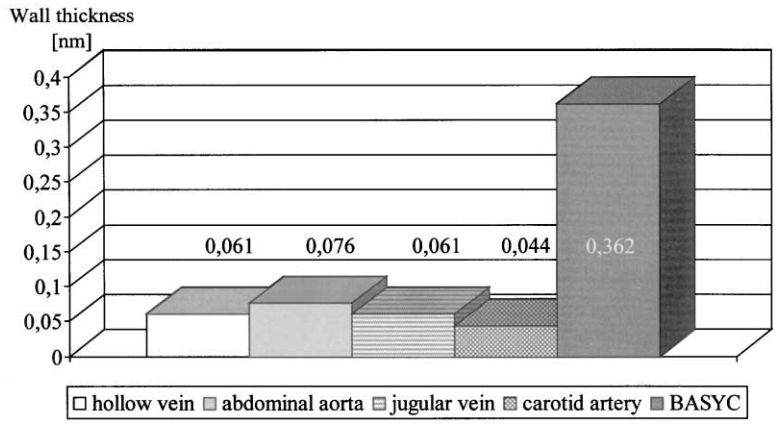

Fig. 28. Average values of wall thickness of several blood vessels of the rat and BASYC®-tubes determined from histological preparations.

of the tensile strength is difficult in respect of the working surface, essential for calculation. However, it could be stated from the experimental microsurgical results that BASYC® in the used dimensions is able to resist the blood pressure of the rat of 0.02 Mpa.

4.3. Examples of application

Due to the previously described properties — special inner surface quality, characteristic consistency, microdimensions, sufficient mechanical strength — BASYC®-tubes prepared by *A. xylinum* AX 5 have been used as artificial blood vessels for microsurgery, as protective cover for micronerve sutures, and in form of a practice model for the training of microsurgical suture techniques in practical courses.

The application of BASYC® as a microvessel endoprosthesis was investigated, e.g. in case of a typical end-to-end anastomosis using the carotid artery of the white rat (Han: WIST). The inner diameter of the designed material was about 1 mm in agreement with the lumen of the vessel. The length of the interposition was in the range 4–6 mm. To demonstrate the implantation area of BASYC® a part of the arterial blood-vessel system of the rat is shown in Fig. 29.

After anesthesia of the animal (intra muscular mixed injection of Ketavet (Ketamin) and Rompun (2-(2,6-Xylidino)-5,6-dihydro-4H-1,3-thiazin-hydrochloride); dosage see Table 4), shave and disinfection of the neck region a skin-incision of 3 cm length was made and subsequently the right carotid artery of the rat was prepared. Fig. 30(1) shows the isolated artery placed on a rubber background. The function of the background material was to keep away connective tissue from the operation area and to give a better contrast. To interrupt the blood flow two single Biemer clips were applied. Between the clips a

Table 4
Dosage of anesthetics for rat anesthesia (BM = body mass)

Ketavet (100 mg/ml)	125 mg/kg BM 0.125 ml/100 g BM
Rompun (50 mg/ml)	16 mg/kg BM 0.032 ml/100 g BM

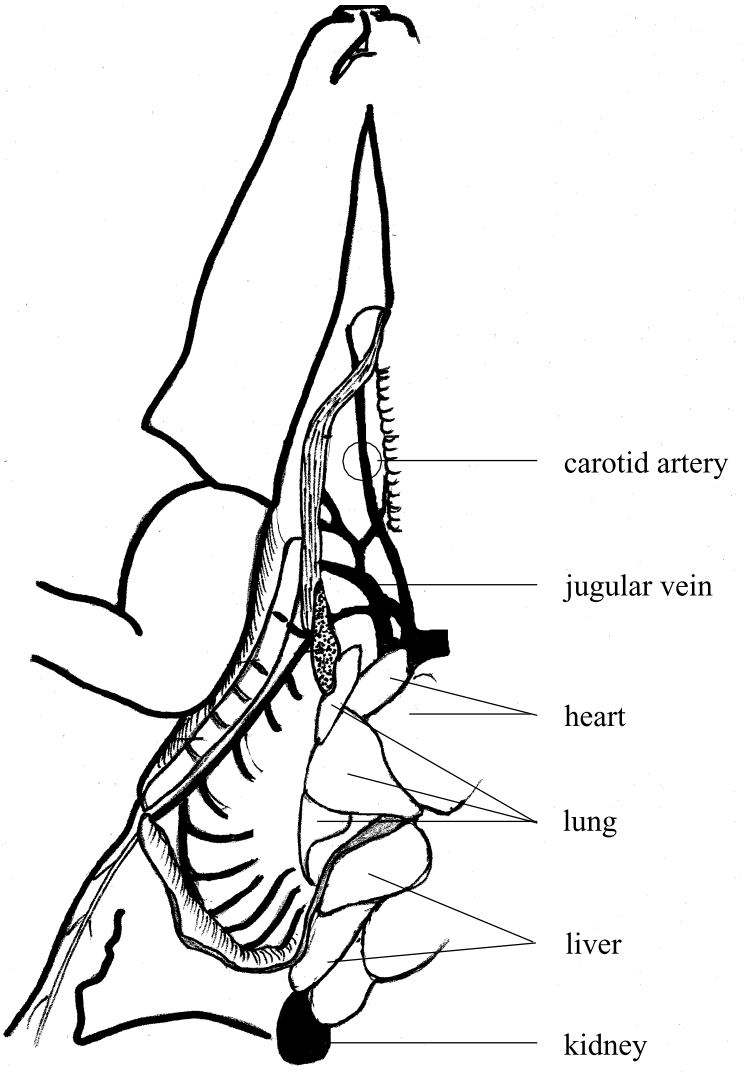

Fig. 29. Part of the arterial blood vessel system of the rat. (Carotid artery as the animal model is marked.)

piece of the vessel of about 5 mm length was resected. A BASYC®-tube of a corresponding size was positioned between the vessel ends and fixed by 6–8 single knot sutures per anastomosis (suture material: Ethilone® BV 6; 10/0). After removal of the vessel clips bleeding that occured was stopped by applying wet swabs, after that the blood perfusion through the BASYC®-tube was proved. Fig. 30(2) shows in detail the two anastomosis areas and the blood flow, visible through the transparent BASYC®-material immediately after the operation. After reposition of the overlaying muscles the wound was closed.

Four weeks postoperatively the experimental animal was anaesthetized again and re-operated. The inspection of the treated vessel showed that the carotid artery–BASYC®-complex was wrapped

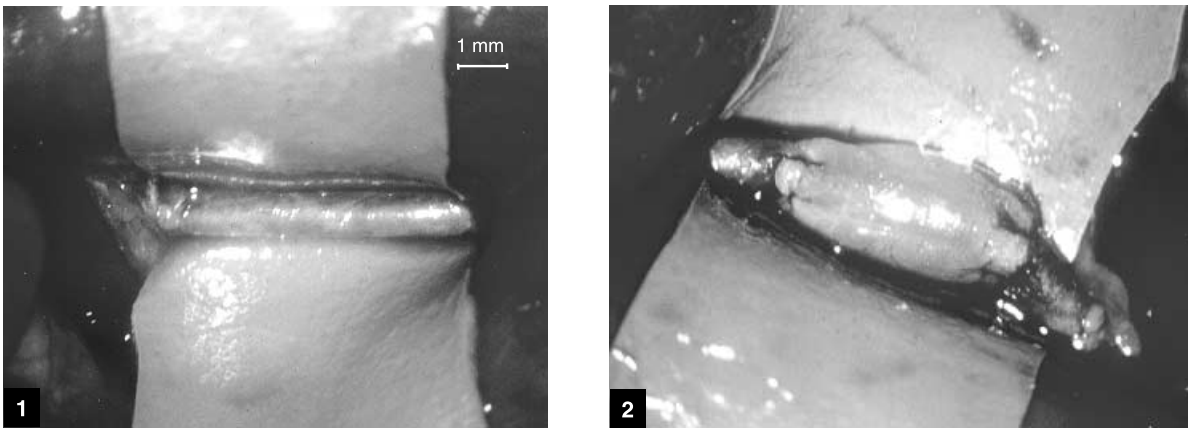

Fig. 30. Steps of implantation of a BASYC®-tube into the carotid artery of the white rat: (1) preparation of the carotid artery; (2) carotid artery with a BASYC®-interposition immediately after operation. Reproduced with permission from Kirshenbaum G, The Gordon and Breach Science Group. Polym News 24 (1999).

up with connective tissue, pervaded with small vessels like vasa vasorum. As shown in Fig. 31 the BASYC®-interposition is completely incorporated in the body without any rejection reaction. The arrows indicate the two anastomosis areas. Using the known Buncke's 'radical pressure test' [122] the patency of the vessel was proved. During the postoperative period the animals were not treated

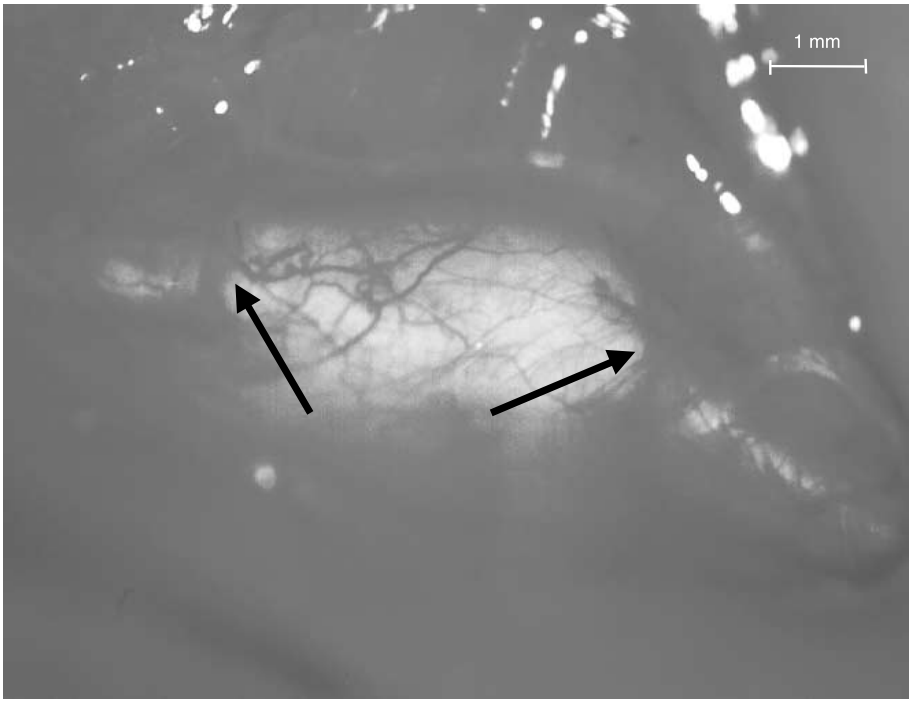

Fig. 31. Carotid artery-BASYC®-complex 4 weeks postoperatively. (→ anastomosis areas).

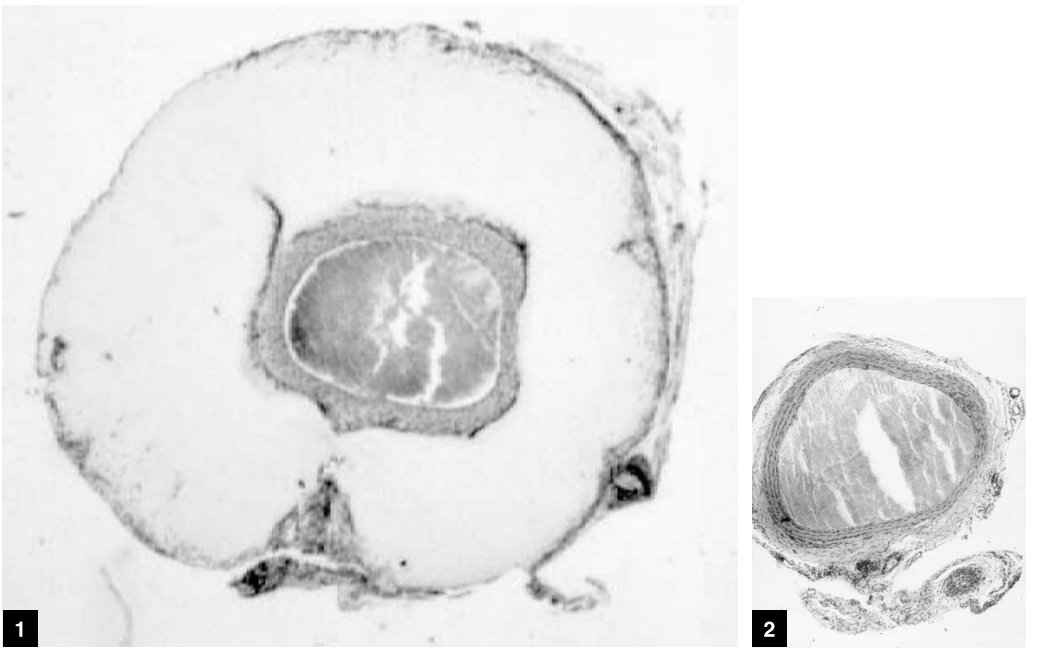

Fig. 32. Histological preparation (H/E staining): (1) BASYC® in the middle region of the interposition 4 weeks after implantation in the carotid artery of the rat; (2) untreated carotid artery of the rat. Reproduced with permission from Kirshenbaum G, The Gordon and Breach Science Group. Polym News 24 (1999).

with anticoagulation agents. Preparations and anastomoses were performed on 30 rats of the WISTAR-strain under an operation microscope (Fa. ZEISS, Germany) with a magnification of 16–25.

All interpositioned BASYC®-tubes had a patency rate of 100% over the entire investigation time provided that the anastomosis is sufficient. There were no signs of coagulation or proliferation.

4.4. Histological investigations

For more detailed information about blood- and tissue compatibility of the cellulosic tubes the carotid artery–BASYC®-complex (BASYC®-prosthesis including the proximal and distal vessel stumps) have been removed and prepared histologically. After dewatering, embedding in paraffin and cutting of the preparations the 7 μm cuts were stained with hematoxylin–eosin (HE) in order to mark cell nuclei in the living tissue. This method allowed to characterize and compare the cross-sections of BASYC®-interpositions and untreated carotid arteries of the rat under light microscopic conditions.

The survey of the outer surface resulted that the whole BASYC® implant was wrapped up with connective tissue. Fig. 32(1) shows that it filled in any impressions of the BASYC® material or unevenness' in the area of anastomosis but never penetrates the material.

The figure also demonstrate that the inner surface of the BASYC®-tube is completely covered with well orientated endogenous cells. From this point of view we assume a 'vitalization' of the artificial endoprosthesis by forming a regular vascular wall inside the BASYC®-material. The orientation of the cells in the direction of the blood flow correlates with the fact that cell functions of several kinds of cells, e.g. endothelial cells, are influenced by their mechanical environment [123]. The vessel region distally to

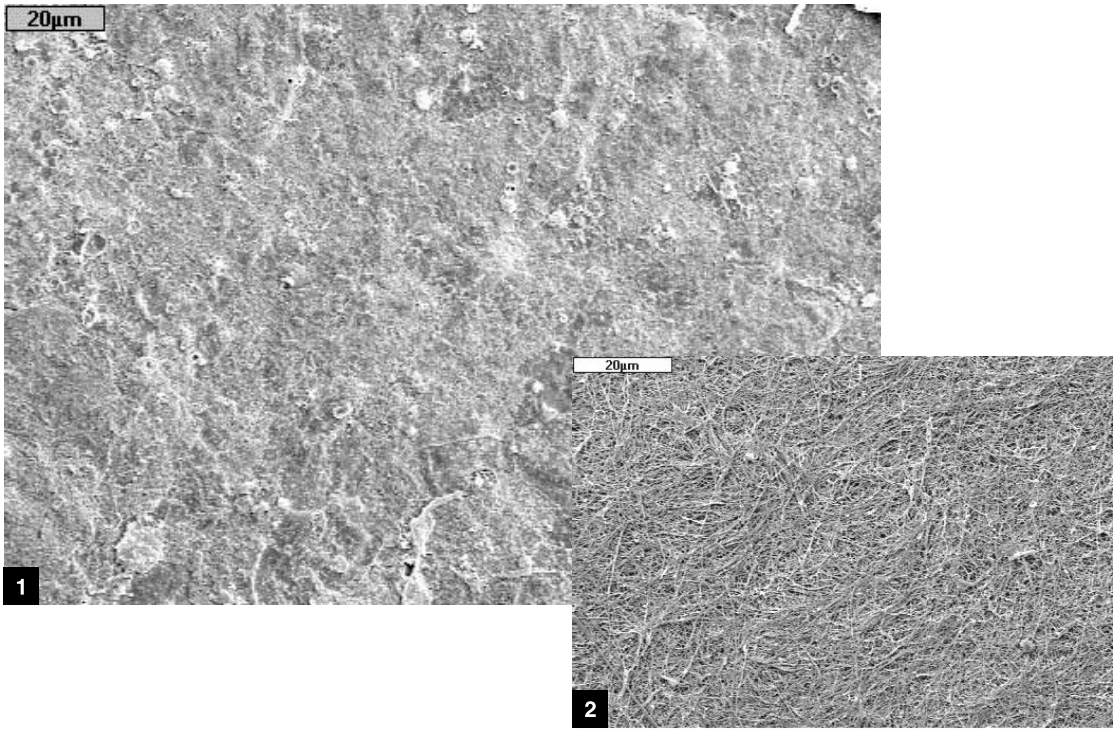

Fig. 33. Scanning electron micrographs (sample preparation: critical point drying) of inner surface areas: (1) BASYC® in the middle region of the interpostion 4 weeks after implantation in the carotid artery of the rat; (2) BASYC® before incorporation.

the second anastomosis with BASYC® shows no alterations and is identical with the control vessel — the untreated carotid artery of the contralateral site (Fig. 32(2)).

Scanning electron microscopic investigations of transversal dissected carotid artery–BASYC®-complexes confirm the results of the light microscope investigation — the complete overlay of the inner surface of the cellulosic material with endogene cells 4 weeks after interposition of BASYC® in the carotid artery of the rat and moreover the areas of the anastomosis (Fig. 33(1). Both the suture line and the suture material were not visible under the cell layer. In comparison with pictures found in literature [124] we assumed the formation of endothelial cells, closely arranged to each other. The previously described and characterized nano-structure network of the cellulosic prosthesis (Fig. 33(2), (see Section 2.3)) is no more visible. Contrary to these results, 4 weeks after the end-to-end anastomosis of the carotid artery of the rat without BASYC® the anastomosis areas were not completely covered with endothelium on the inside of the vessel. Suture material was still visible. The endothelial cells close to the anastomoses were loosely arranged, and the areas of ananstomosis are clearly thrown up.

4.5. Immunohistological investigations

To prove the presumption that the newly built endogenous cells in the BASYC®-tubes are real endothelial cells a special histochemical technique was used. This immunohistochemical method was

based on an antigene–antibody-reaction with a color reaction of the marked cells visible under the light microscope.

Following the principle that the healing of ill and the receipt of healthy tissue around an implant base on cellular and molecular interactions at the tissue–implant interface, our actual research is directed to detection and understanding of these processes.

It is known that fibronectin can be found in regions, which are active concerning wound healing processes and building new vessels. It is said to be a 'guide track' for fibrosis and the building of regenerative tissues. Fibronectin is described as a structure and adhesive molecule [125–129]. We will try to show fibronectin by histological staining in different times after interposition of BASYC®.

At present, in vitro tests are carried out to examine the growth of cell cultures on the inner surface of BASYC®-tubes. Our efforts are concentrated on the explanation of the processes of cell adhesion, proliferation and sprouting out.

5. Further applications of BASYC®in microsurgery

5.1. Micronerve surgery

Additional to the microvessel surgery BASYC® is useful in different types of microsurgical applications.

An important area is the surgery of nerves. A covering of the anastomosis for the protection of the sutured fascicles and the prevention of a growing of connective tissue into the gap between the anastomosed nerve ends is meaningful.

In an experiment with white rats (Han: WIST) the sciatic nerve was prepared and dissected. The BASYC®-tube was pulled over one of the nerve stumps and subsequently the anastomosis of the fascicles was carried out by single knot sutures. The BASYC®-cover was positioned directly over the anastomosis area and fixed by two holding sutures. The anastomosis of the nerve is visible through the transparent BASYC®-cover (straight arrow, Fig. 34(1)). Operation conditions and the animal strain were identical with those of the experiments in microvessel surgery (see Section 4.3).

After the observation time from 4 to 26 weeks postoperatively the BASYC®-tube was covered with connective tissue and small vessels within (dotted arrow, Fig. 34(2)). Neither an inflammation reaction nor an encapsulation of the implant was to be observed. The regeneration of the nerve functions was improved after 10 weeks compared to an uncovered anastomosed nerve. As an indicator of the regeneration of the nerve function the increasing muscle weight of the extensor digitorum longus muscle (EDL) of the rat was determined. Another indicator was the reappearance of acetylcholine as the transmitter of nerve impulses to the executive organ. It was indirectly detected by determination of the activity of acetylcholinesterase using GOMORI's method of acetylthiocholineiodid staining [130].

Using the above mentioned animal model (sciatic nerve/EDL) in another experiment BASYC® served as a drug depot for a substance with neuroregenerative properties (experiments were carried out in cooperation with the Max-Planck Research Unit, Enzymology of Protein Folding, Halle/Saale, Germany) [131]. The tested substance was cycloheximide-*N*-(ethyl ethanoate), satisfying the required criteria for a differential cycloheximide-derived FKBP inhibitor (FKBP = FK506-binding protein). Immediately after nerve reconstruction the substance was applied to the anastomosis and stored in the BASYC®-cover. For comparison a placebo (the dipeptide Ala–Ala–OH) was examined likewise. At the end of the

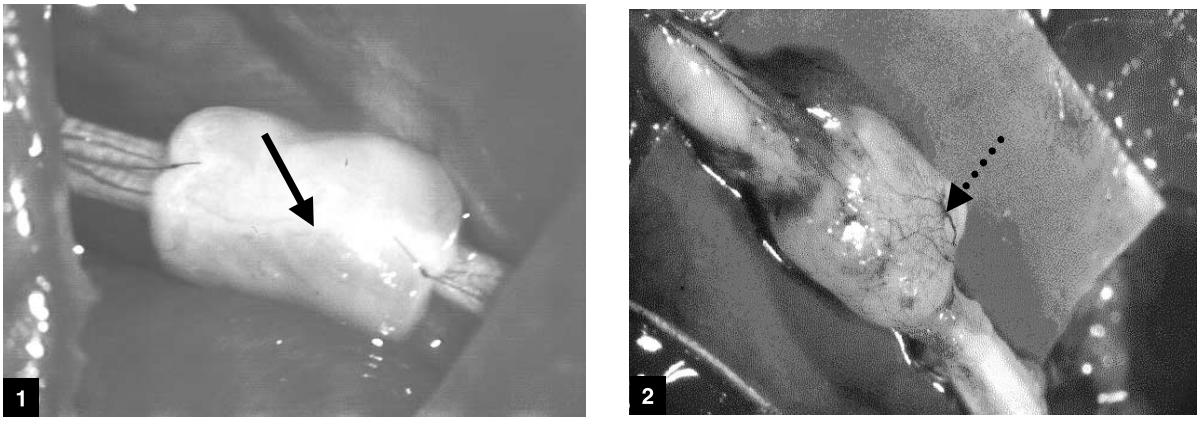

Fig. 34. Application of a BASYC®-tube in micronerve surgery: (1) sciatic nerve of the rat with a BASYC®-tube as protective cover immediately after operation; (2) sciatic nerve of the rat with the BASYC®-cover 10 weeks after operation.

observation time (8 and 10 weeks) the EDLs were removed and the muscle weight was determined. In comparison to the placebo the tested substance caused an earlier return of innervation, measurably at the muscle weight. In addition to the method of the muscle weight determination, the functional recreation of the paralyzed legs was evaluated by the observation of the walking behavior. These results correspond with those of the muscle weight determination. In comparison to placebo treated rats, the walking behavior of cycloheximide-derivate treated animals for eight weeks after nerve injury was better than the 10 weeks treated.

5.2. Training of microsurgical techniques

The successful application of the microsurgical operation technique is impossible without an intensive microsurgical training. For reasons of animal protection it is necessary to use simple models consisting of rubber membranes or plastic tubes in the first step. These models are very easy to handle but they do not permit however a close-to-reality training of microsurgical anastomosis techniques. Besides the preparation of a sufficient anastomosis the careful handling with the living tissue is an important requirement for the success of the microsurgical operation. Bruising and stretching of the microvessel are traumatizing and result in damages of the vessel wall [132]. They are limiting factors for a successful anastomosis together with lesions caused by temporary vessel occlusion with microclips [133–137].

Under this criterion we developed a new model, which enables a training of all microsurgical techniques by copying conditions in the living organism.

The BASYC®-tubes are integrated components of the model system. The practice unit permits an optimal and close-to-reality training that means manipulation and anastomosing in a wet milieu. An incorrect handling with microsurgical instruments results in visible changes of the shape of the BASYC®-tube like in a natural vessel.

Consequently, the model disciplines to a careful preparation and operation.

In Fig. 35 some techniques are shown. In Fig. 35 an end-to-end anastomosis (1) and (3), an

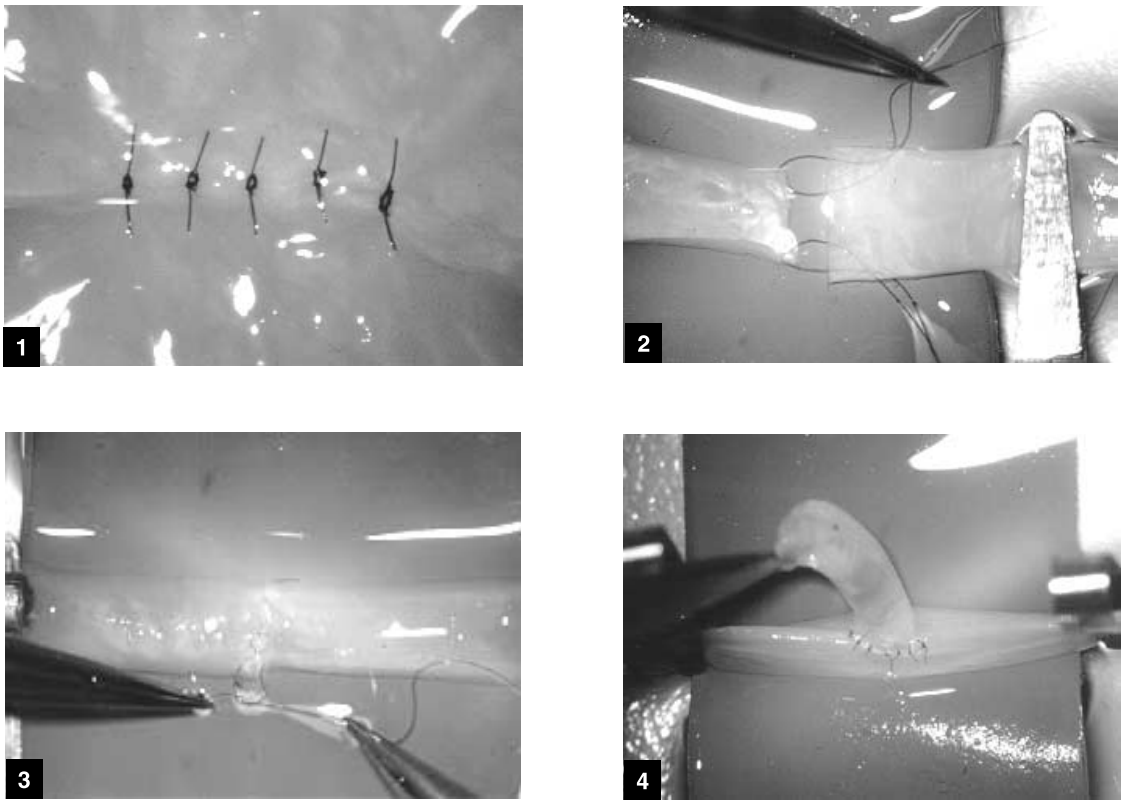

Fig. 35. Training of different microsurgical suture techniques with the help of BASYC®: (1) end-to-end anastomosis (single knot suture); (2) end-in-end anastomosis; (3) end-to-end anastomosis (continuous suture); (4) end-to-site anastomosis.

end-in-end-anastomosis (2), and an end-to-side anastomosis technique (4) is demonstrated. Fig. 35(1) and (3) shows the typical single knot suture and a continuous suture, respectively. The training model is used successfully in practical microsurgical courses of the Clinic of Maxillofacial and Plastic Surgery of the University of Jena and reduced the number of necessary experimental animals.

6. Discussion and conclusions

Due to rising life expectancy and advances in the implant technology the number of implantations in human medicine increases continuously. Based on the improvements in the field of microscopy, microsurgical instruments and suture material, the microsurgical technique has been developed to an operation method, which permits most complicated interventions at finest structures of the human organism. It is applicable in all surgical fields. In most cases this technique offers the only possibility of functional and esthetic re-creation of the patient and restore its health and life quality.

Success or failure of a microsurgical operation primarily depends on a perfectly performed microsurgical anastomosis. One of the reasons for failures of such complicated, risky, and expensive procedures is the long operation time caused by problematic conditions in the recipient area — difference of

the inner diameters of the anastomosed vessels, lesions at the blood vessels after radiotherapy or accidents. In all these cases microvessel interpositions are necessary.

Since the beginning of the last century autogenous vein transplants have been used for reconstruction of arteries [138,139]. The preparation of these vessels requires a considerable time exposure connected with an additional operation.

For a long time scientists of the whole world have looked for alternatives. The research is directed on the development of implant materials, which will be integrated without complication into the tissue of the patient and maintain the close connection over the time. Thereby clinical problems such as loosening of the implants, destruction of tissue around the implants, pain, as well as surgical revisions could be avoided. Especially implantation materials, which are in a direct contact with blood, have to meet a lot of requirements: bio- and blood compatibility, mechanical strength against the occurring blood pressure, tightness to the blood and its constituents, and sterilizability. In addition, the healing process taking place on the inner and the outer surface of the artificial vessel is very different. The inner surface of the biomaterial should not stimulate adhesion of cellular blood components but should be covered with endothelial cells, whereas the outer surface of the prosthesis should be wrapped up with connective tissue.

Commercial prosthesis-materials like polytetrafluoroethylene/PTFE (Teflon®, Gore-Tex®), polyethylene terephthalate/PET (Dacron®), polyurethane or silicone which are applied in heart, vessel and thorax surgery with an inner diameter of the vessels greater than 3 mm are unsuitable for microsurgical requirements. According to the definition microvessels have an inner diameter smaller than 3 mm. In a lot of publications experimental studies to short- and long-time behavior of synthetic small caliber prosthetic grafts was reported. Because of the high risk of thrombosis these microvessel grafts until today have not been used in clinical application [140–149]. Reasons for the failures are seen in a hypoplasia of the intima in the region of anastomosis, an extensive perivascular fibrosis or a high thrombogenity of the implanted material [150,151]. Greisler and co-workers described that fabrication of polypropylene into an arterial substitute might result in an efficacious prostheses because of the physical properties (e.g. high tensile strength) and relatively inert behavior of the material. After implantation in the abdominal aorta of dogs the authors determined a better late patency for polypropylene grafts (4 mm I.D. × 50 mm in length) than for Dacron and ePTFE. In one month, a confluent endothelialized surface was seen in all explants [152].

Expensive attempts were carried out to optimize the inner surface of the implant materials. The scientific work is concentrated on the development of suitable coatings based on proteins like collagen [153,154], gelantine [154–156] and albumin [154,157,158] as well as chitosan-poly(vinylalcohol) blends [159], heparin [150] or hyaluronic acid [160]. Further investigations include the transplantation of endothelial cells on the surface of the endoprostheses [161–169]. In addition, the fixation of cell receptors or growth factors [170–173] in coating materials are interesting developments in the field of biomaterial research.

Another part of the investigations is the in vitro synthesis of vessels by tissue engineering starting from co-cultures of vessel-forming cells and smooth muscle cells. The combined culture of both kinds of cells as well as flux cultivation conditions improves the properties of the formed vessels, e.g. the physiological construction and the stretching stability [123].

It can be estimated that no suitable prosthesis material has been available until now that can be used in the clinical microvessel surgery. At present autogeneous vein interpositions are still the best alternative for reconstruction of damaged vessels in human medicine.

We have developed a prosthesis material, which can meet a major part of the above-mentioned requirements.

Bacterial cellulose is produced by a biotechnological method starting from glucose during a short period of 10 days. A great advantage of the biopolymer consists in its moldability in situ. The unique supramolecular structure leads to a remarkable combination of high crystallinity, important hydrophilicity, unexpected smooth inner surface and sufficient mechanical strength in its wet state.

Our animal experiments demonstrate that structure and properties of the cellulosic microvessel endoprosthesis material cause a rapid adhesion and an optimal coating with endogeneous cells in the living body [174–188].

Forming a new vessel wall at the inside of the BASYC®-tube similar to the original vessel means a vitalization of the cellulosic biomaterial in animal and could be explained as a special kind of tissue engineering taking place in vivo. Tissue engineering means the construction of living substitution tissue by combination of cells and biomaterials. The biomaterial is populated in vitro with cells, and during the development of the tissue in the culture, the matrix material is decomposed. Ideally, the final product is a tissue with the original shape of the matrix. The properly matching new tissue should be implanted in the defect of the patient, integrated in the endogenous tissue, and took over the desired function [123].

The results of first experimental applications in microvessel surgery, micronerve surgery, and microsurgical practice demonstrated the remarkable advantages of this bacterial polysaccharide: handling in an easy and practice-orientated way, blood and tissue compatibility, consistency comparable to those of the blood vessel, vitalization in the living body. From this point of view, bacterial cellulose in a biomaterial with extraordinary properties meets the preconditions to develop ‘different replacements’ applicable not only in the microvessel surgery.

The patented name ‘BASYC’ designates in a narrow sense the designed bacterial cellulose product.

7. Outlook

Further investigations to test the BASYC®-tubes as microvessel prosthesis for veins are under progress. First results of the implantation of BASYC®-material into jugular veins of 10 white rats (Han: WIST) showed no complications like wall-proliferation, stenosis or thrombosis. A normal blood flow through the interposition as well as the formation of connective tissue around it were detectable 4, 12 weeks, respectively, after the operation [186].

Besides the promising use of BASYC® in the experimental microsurgery we see the possibility of application of bacterial cellulose as a soft tissue substitution material in different medical fields. The internal medicine, urology, gynecology, otolaryngology, maxillo-facial or plastic surgery could be potential users of the designed biopolymer. For this reason hollow cellulose fibers with different inside diameter and different wall thickness were prepared in microdimensions. Fig. 23 shows a collection of BASYC®-tubes.

The immobilization of biological active substances within the cellulosic network structure to optimize healing and regeneration processes as well as to test the potential pharmaceutical suitability of natural or synthetic substances are actual points of our study.

In the field of microvessel surgery, first investigations were carried out to load the BASYC®-tubes with hyaluronic acid, a mucopolysaccharide occurring in the living organism with inflammation inhibiting and antithrombogenous properties.

As mentioned above (see Section 5.1) the rat sciatic nerve neurotomy model [189] was used in combination with BASYC®-covers loaded with a neuroprotective substance [131]. The experimental results demonstrate that this type of BASYC® application is meaningful and further studies with other active substances will be realized.

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