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Manufacturing and microscopical characterisation of polyurethane nerve guidance channel featuring a highly smooth internal surface

G. Soldani[a,]*, G. Varelli[a], A. Minnocci[b], P. Dario[b]

[a]*Istituto di Fisiologia Clinica del CNR, Via Trieste 41, 56100 Pisa, Italy*
[b]*Scuola Superiore di Studi Universitari e di Perfezionamento S. Anna, Pisa, Italy*

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Abstract

The present study demonstrates the possibility of manufacturing polyurethane [ChronoFlex® (CF)] nerve guidance channels (NGCs) featuring a highly smooth internal surface. Comparative SEM and AFM observations prove marked differences between the internal surface microgeometry of Silastic and CF channels. SEM of CF samples shows a surface with no detectable roughness, while Silastic channels show transversal rows along the entire surface. AFM digital image of Silastic samples show a surface with a rough microgeometry defined by a tridimensional pattern with peaks up to 1400 nm. AFM digital image of CF samples show, indeed, an essentially flat microgeometry with the highest level at 545 nm. These preliminary results suggest that the association of an innovative sequential deposition manufacturing technique with the new CF polyurethane may produce NGCs with a smoother surface microgeometry, in comparison to NGCs obtained from commercial Silastic tubes. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Silastic; Polyurethanes; SEM; AFM; Microgeometry; Nerve guidance channels

1. Introduction

The peripheral nervous system (PNS) has regenerative capability [1]. After an injury axons can repair the gap between the stumps. The present techniques of reconstructive surgery allow to repair traumatic lesions following nerve resection without loss of tissue. The technique resides in the realignment of the nerve and in its suturing while attempting to respect its fascicled geometry. In cases of loss of tissue the following techniques can be employed: nerve mobilisation, positioning of joints, sutures under tension and a transplant [2]. However, these techniques do not always provide satisfactory results, e.g.: in the event of the nerve mobilisation and of the suture under tension, which are used when the loss of tissue is slight, the strain on the nerve cable produces impairment of axonal regeneration [2]. If the loss of tissue is severe the treatment of the lesion is still a challenge for the surgeon, and, therefore, to overcome the gap between the stumps, experimental surgical techniques take into consideration the nerve grafting supported by nerve guidance channels (NGC) made from different materials [2]. Since 1880 research in the field of neuroprostheses has shown interest in several materials shaped as NGC to connect the distal and the proximal stump of injured nerves. Initially, materials investigated were of a biological source, such as bone, veins, artery, fat, epineurium, trachea, muscle, dura mater and collagen [3]. More recently, biodurable materials such as Silastic (silicone elastomer), Millipore®, polyethylene (PE), acrylic copolymers and polytetrafluoroethylene (PTFE) [4–10], and bioresorbable materials such as polyglycolides, polylactides and other polyesters have also been considered [11]. However, in all cases, these materials have displayed variable degrees of success in bridging transected nerves, and the newly formed nerves were morphologically quite different from normal peripheral nerves [10, 11]. NGCs made of fluorinated polymeric materials such as PTFE and polyvinylidene fluoride (PVDF) may show interesting features of long-term biostability, but their stiffness, poor suturability and high resistance to the needle penetration make them difficult to handle from

*Corresponding author. Fax: 0039 50 503596; e-mail: soldanig@nsifc.ifc.pi.cnr.it.

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a surgical point of view. Silastic tubing is reported to support regeneration over large gaps in several mammalian species, and due to its long-term biostability and mild inflammatory reaction have been used, on a very limited basis, in the clinical repair of severed nerves [11]. The limitation in the use of silicone NGC is due to the poor morphological and functional outcome of the regenerated nerve cables. This might be related to the relatively rough topographical morphology (microgeometry) of the inner surface of commercial Silastic tubes rather than to material properties by itself. In this respect, a relative study reports that nerves grow better in NGCs having a smooth internal surface as compared to identical NGCs featuring a relatively rough internal surface [12].

Regarding the above-mentioned findings, the purpose of the present study was to manufacture and characterise polyurethane NGCs showing a very smooth internal surface.

2. Materials and methods

2.1. NGCs manufacturing

NGCs were manufactured by a sequential 'dipping' and 'spray, thermal-heating' (STH) technique (1), using as a biomaterial the ChronoFlex® (CF) (AL-80A) (PolyMedica, Inc., Woburn, MA, U.S.A.), a new family of thermoplastic polyurethane (PU) elastomers claimed to sustain in vivo biodegradation [13–14]. Both to facilitate channel removal and smoothening of its internal surface, a channel mould of borosilicate glass capillary (2 mm OD, 10 cm long) was dip coated with a thin layer of Polyvinylalcohol (PVA). Subsequently, the PVA coated mould was covered with multiple layers of CF by a 'spray-machine' equipment (Kontron Instruments S.p.A. – Milano, Italy) [15] and according to the STH technique. Finally, the CF channel was removed from the mould and treated. Procedural steps are reported as follows:

2.1.1. Dipping technique

The PVA coating of the glass capillary was made by a dipping apparatus, that plunged the glass capillary in a 7% solution of PVA in water, at 60°C (Fig. 1). After dipping the capillary was placed under a glass-bell for 24 h at room temperature.

2.1.2. STH technique

The PVA coated mould was mounted in place on the spray-machine equipment by two special mandrel. A 1% solution of CF in tetrahydrofurane (THF) was deposited with a spray-gun onto the PVA coated mould at a flow rate of 1 ml min^{-1}. The 'spray-machine' operates an alternate and continuous translation of the spray-gun while the channel mould rotates (Fig. 2). The translation speed, the polymer solution flow rate, the working pressure of the spray-gun, and the rotation speed of the channel mould were all controlled by a computer. The

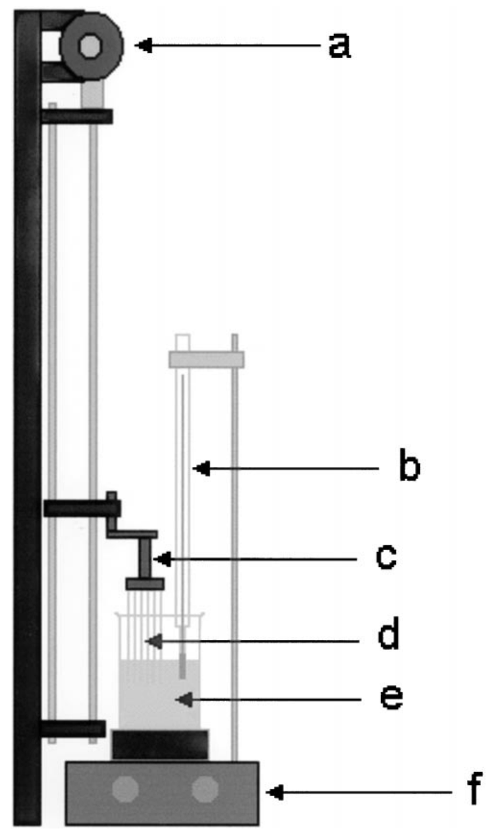

Fig. 1. Schematic representation of the dipping apparatus: (a) motor; (b) temperature controller; (c) multidipper adapter; (d) glass capillaries; (e) PVA solution; (f) hot plate.

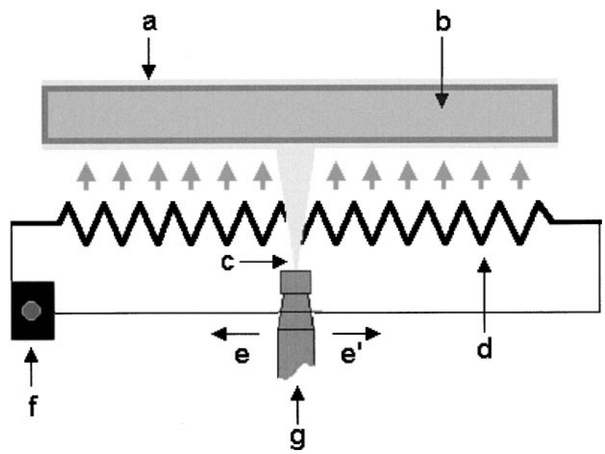

Fig. 2. Schematic representation of the 'spray, thermal-heating' (STH) apparatus: (a) polymer deposition; (b) glass capillary mandrel; (c) polymer solution jet; (d) heating element; (e) alternating movement; (f) temperature controller; (g) spray-gun.

entire process was carried out in a chemical fume hood. Initially, the temperature in the proximity of the mould was maintained below the glass transition temperature (t_g) of PVA and, subsequently, after a certain number of CF coatings, the temperature was raised to about 70°C to evaporate completely the THF solvent in which the CF was dissolved. At the end of the STH procedure the CF/PVA coated mould was removed from the 'spray-machine' and placed under a glass-bell for 24 h at room temperature to allow drying of the polymer [1].

2.1.3. CF channel removal from the mould and annealing

The CF/PVA coated mould was placed in water at 60°C to dissolve the thin layer of PVA until the CF channel was free to move out of the glass capillary mould, afterwards the CF channel was annealed in a vacuum oven at 60°C for 4 h. The 2 mm ID, 10 cm long CF tube so obtained was cut in a 1 cm long NGCs. Samples were used both for Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) to evaluate the internal surface microgeometry.

3. SEM

Both ultrasonically cleaned Silastic and CF-NGCs samples were examined. Samples were mounted on aluminum stubs with silver print, coated with 20 nm of gold and observed in a Philips SEM 515. Pictures were taken at an accelerating voltage of 10 KV.

4. AFM

Both ultrasonically cleaned Silastic and CF-NGCs samples were examined. Channels samples of approx. 5–6 mm in length, were cut lengthways with a lancet, taking care not to damage the inner surface. Then they were opened and glued by the outside surface onto the sample holder using cyanoacrylic adhesive and finally cleaned with a nitrogen flow. Images were obtained in air with a Topometrix TMX 2000 Discoverer in the constant force (contact) mode. Constant force is used when there is a significant change in the height of features over the scan area. Scanning was performed with an image size of $5000\ nm \times 5000\ nm$, acquiring 400×400 pixels with a scan rate of $15000\ nm\ sec^{-1}$.

5. Results

The sequential dipping and STH technique allows to manufacture NGCs which show excellent characteristics of elasticity, flexibility and low resistance to needle penetration. The CF material is suitable for the STH process due to its aliphatic-based structure which needs to be

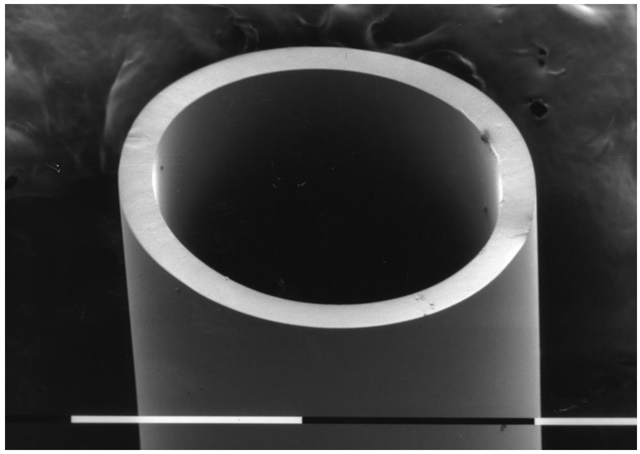

Fig. 3. Tilted SEM view of the cross-section of a Chronoflex channel. Note the smooth appearance of channel surfaces and the thin wall (about 140 μm) (marker = 1 mm).

thermally annealed to allow surface crystallisation of the hard segments [13]. The fine polymer deposition made in a controlled temperature environment and the final thermal treatment produces a thin wall (about 140 μm), non collapsible and highly transparent NGCs (Fig. 3). Comparative SEM observations between the internal surface of Silastic and CF channels which were not implanted show marked differences. Silastic samples (Fig. 4) show a rough internal surface with transversal rows along the whole surface, while CF samples (Fig. 5) show an internal surface without detectable roughness. AFM shows in more detail the microgeometry pattern of these two materials. AFM digital image of the Silastic sample (Fig. 6) shows a surface with a rough microgeometry defined by a three-dimensional pattern with a plurality of 'valleys' and a plurality of 'rocks-like' solid portions. These solid portions varied in height up to 1400 nm. In addition, a conic structure with an extended base is visible at the top, left side of the picture. The peak of the structure, which has been cut by the image frame, is likely to extend far beyond the 1400 nm value (Fig. 6). The AFM digital image of the CF sample (Fig. 7) shows a surface with an essentially smooth microgeometry defined by no asperity and sparse 'moon-craters' like areas of low depth. The surface is uniformly distributed up to the highest level of the investigated area (545 nm).

6. Discussion

This study demonstrates the possibility of manufacturing, by a sequential dipping and STH technique, NGCs featuring a smooth internal surface. SEM and AFM demonstrate that the internal surface topographical morphology (microgeometry) of Silastic channels is rough, respect to the microgeometry of CF channels which is highly smooth. It could be possible that in Silastic tubes

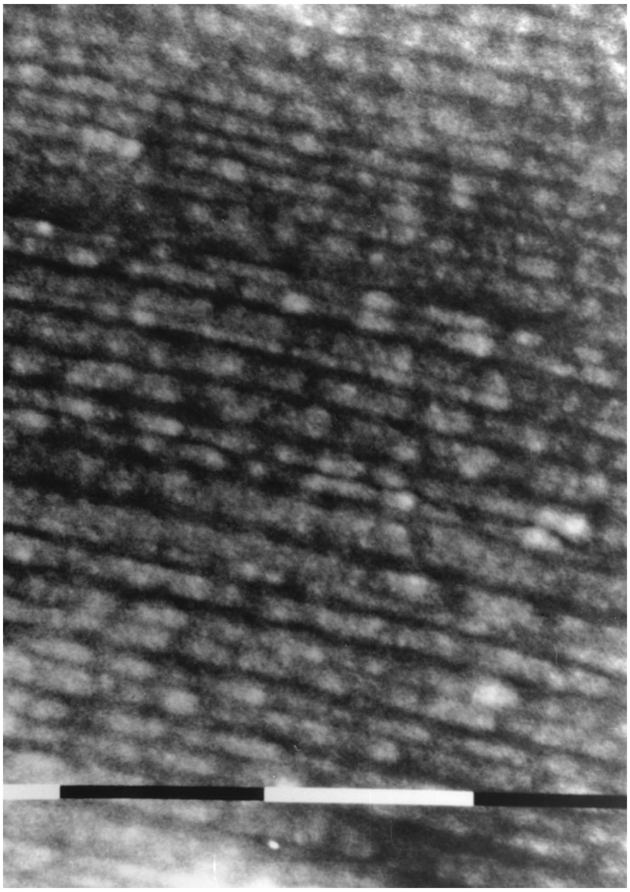

Fig. 4. High magnification SEM of the internal surface of a Silastic channel. Transversal rows are visible along the entire material surface (marker = 10 μm).

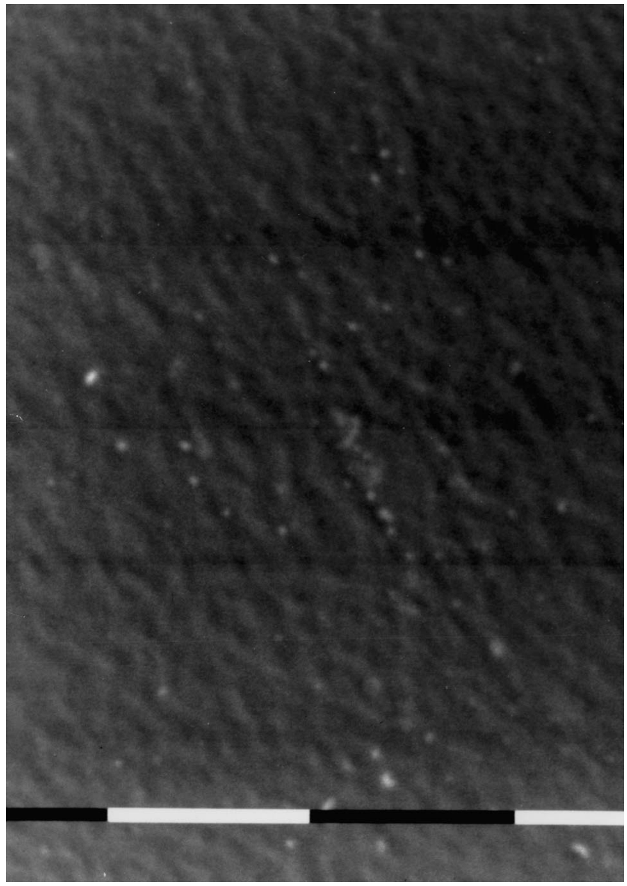

Fig. 5. High magnification SEM of the internal surface of a Chronoflex channel. No detectable roughness are present on the surface (marker = 10 μm).

rows form at the time of manufacturing as a consequence of some geometrical irregularities present in the extrusion nozzle. Differences in surface microgeometry are likely to produce different nerve cable reactions as regards the contacting biomaterial surface and, therefore, a different nerve regeneration outcome can be expected. It has been demonstrated that regenerating peripheral nerves is modulated by the surface microgeometry of synthetic NGCs [16]. Rough inner surface tubes elicit an intense inflammatory response in comparison with tubes featuring a smooth inner surface. Macrophages, fibroblasts, red blood cells and degenerating white blood cells are found in the rough tube. A fibrin matrix arranged with a longitudinal orientation, which favours the regeneration of the nerve cable, is found in the smooth tube. In this case, the nerve cable is centred, free from attachment to the tube wall and contains blood vessels, also the axons are grouped in microfascicles and surrounded by an epineurium [12]. It may be hypothesised that the microgeometry of the inner surface of the NGC modulate the nerve regeneration process by altering the protein and cellular components of the regenerating tissue bridge. Channels with a rough inner surface may alter the mechanism of fibrin gel formation. The fibrin, instead of forming a longitudinally oriented loose matrix connecting the nerve ends, draw together by ‘syneresis’ the particles of the dispersed phase of the gel, with separation of some of the disperse medium and shrinkage of the gel. As a result, cells migrating in the fibrin gel from the nerve stump disperse in the entire lumen rather than form a discrete central structure [11, 12]. In general, SEM and AFM morphological evaluation allows, from different levels, to obtain images of topography and microgeometry of biomaterials, which permit a better understanding of cellular dynamics in the ongoing presence of biomaterials. In our study SEM and AFM have been used to estimate surface asperities down to a nm scale, which may give an indication of the expected performance of the NGC in vivo. Necessarily extensive studies in a suitable animal model need to be performed to evaluate the morphology and functionality of the regenerated nerve cable. However, these preliminary results suggest that the association of an innovative channel manufacturing technique, such as the sequential dipping and STH, with the new CF polyurethane may produce NGCs with a smoother surface microgeometry in

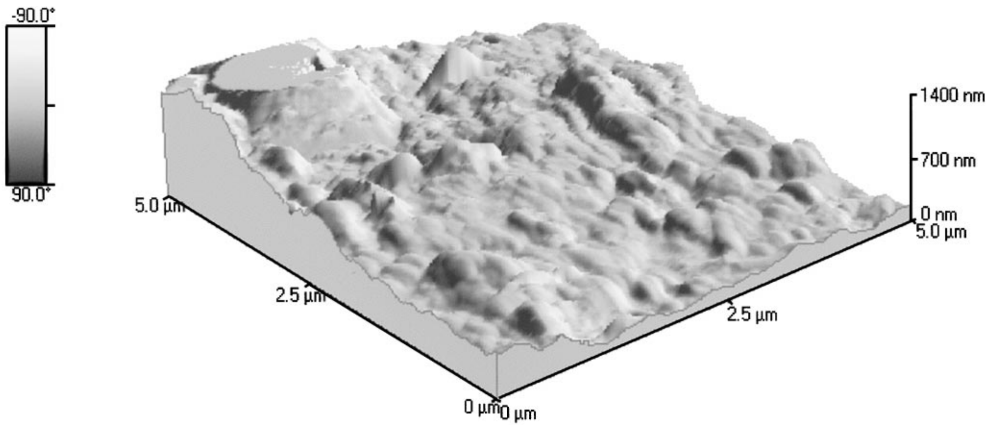

Fig. 6. Digitalised AFM image of the Silastic channel internal surface. A very rough microgeometry defined by a three-dimensional pattern with a plurality of 'valleys' and a plurality of 'rocks-like' solid portions is visible (5 µm × 5 µm).

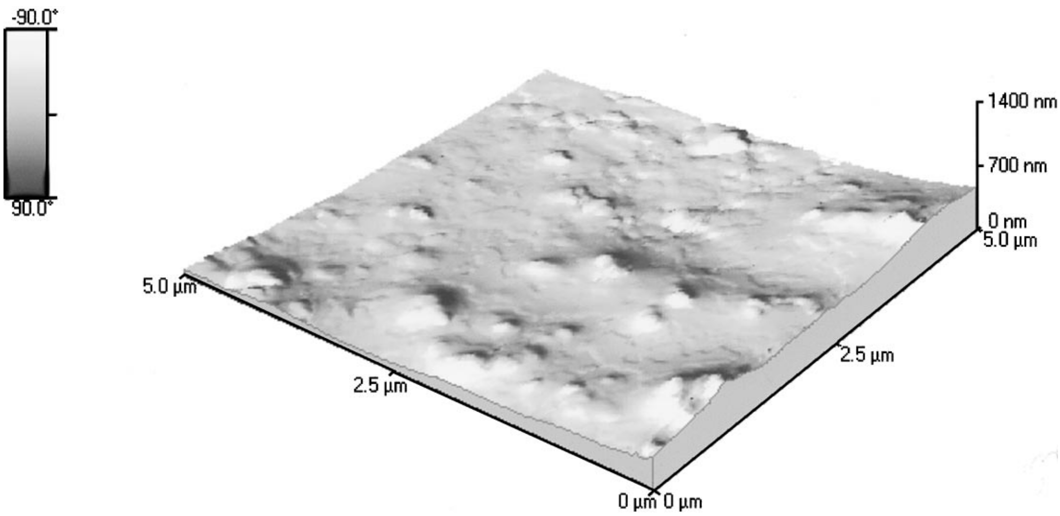

Fig. 7. Digitalised AFM image of the Chronoflex channel internal surface. An essentially smooth microgeometry defined by no asperity and sparse 'moon-craters' like areas of low deepness is visible (5 µm × 5 µm).

comparison to NGCs obtained from commercial Silastic tubes.

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References

[1] Soldani G, Cocco M, Dario P, Sebastiani L, Varelli G. Towards the development of an integrated neural connector: technologies to fabricate nerve guidance channels and silicon dice. J Micromech Microeng 1995;5.

[2] Gelberman RH. Operative nerve repair and reconstruction. JB Lippincott Comp 1991.

[3] Weiss P. The technology of nerve regeneration: a review. Sutureless tubulation and related methods of nerve repair. J Neurosurg 1944;1.

[4] Ducker TB, Hayes GJ. Experimental impovements in the use of Silastic cuff for perpheral nerve repair. J Neurosurg 1968;28.

[5] Bassett CAL, Campbell JB, Husby J. Peripheral nerve and spinal cord regeneration: factors leading to success of a tubulation technique empolynig Millipore. Exp Neurol 1959;1.

[6] Gibby WA, Koerber HR, Horch KW. A quantitative evaluation of suture and tubulization nerve repair techniques. J Neurosurg 1983;58.

[7] Ducker TB, Hayes GJ. Experimental improvements in the use of Silastic cuff for peripheral nerve repair. J Neurosurg 1968;28.

[8] Valentini RF, Sabatini AM, Dario P, Aebischer P. Polymer electret guidance channels enhance peripheral nerve regeneration in mice. Brain Res 1989;480.

[9] Aebischer P, Valentini R, Dario P, Domenici C, Galletti PM. Piezoelectric guidance channels enanche regeneration in the mouse sciatic nerve after axotomy. Brain Res 1987;463.
[10] Valentini RF, Aebischer P, Winn SR, Kunz S, Galletti PM. Peripheral nerve regeneration through guidance channels: the effect of channel composition. Life Support Systems 1986;4.
[11] Valentini RF. In: Bronzino JD, editor. The biomedical engineering handbook. Vol. XXXII, Boca Raton (FL): CRC Press, 1995; 1985–96.
[12] Aebischer P, Guénard V, Valentini F. The morphology of regenerating peripheral nerves is modulated by the surface microgeometry of polymeric guidance channels, Brain Res 1990;531.
[13] PolyMedica Industries Inc., ChronoFlex® technical sheet MA: Woburn.
[14] Szycher M, Reed AM, Siciliano AA. In vivo testing of a biostable polyurethane, J Biomat Appl 1991;6.
[15] Soldani G, Panol G, Sasken HF, Goddard MB, Galletti PM. Small diameter polyurethane-polydimethyl-siloxane vascular prostheses made by a spraying, phase-inversion process. J Mat Sci 1992;3.
[16] Aebischer P. In: Bronzino JD, editor. The biomedical engineering handbook. Vol. XXXII, Boca Raton (FL): CRC Press, 1995; 1755–73.