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Evaluation of the tissue reaction to a percutaneous access device using titanium fibre mesh anchorage in goats

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The tissue reaction to a percutaneous access device, applicable as a carrier for an implantable glucose sensor, was evaluated in goats. Titanium fibre mesh structures were used for anchorage of the device in superficial as well as deeper soft-tissue locations. The percutaneous part was subcutaneously anchored with a fibre mesh sheet. The distal part was placed intraperitoneally and anchored in deeper soft-tissue layers using a fibre mesh cuff. All implants showed good healing with the surrounding tissue. Histological evaluation showed that the subcutaneous fibre mesh sheets and peritoneal fibre mesh cuffs were filled with immature connective tissue, generally free of inflammation. Problems concerning disconnection of the silicone catheter from the titanium holding element and filling of part of the peritoneal fibre mesh cuff with silicone glue have to be overcome by more appropriate preclinical testing and improved implant design. Our results demonstrate that titanium fibre mesh structures can be used effectively for soft-tissue anchorage of percutaneous access devices. A sufficient ingrowth of connective tissue was obtained in superficial as well as in deeper soft-tissue layers. The access device could have application as a carrier for an implantable glucose sensor for glucose monitoring in different tissue compartments.

1. Introduction

Continuous monitoring of glucose metabolism by an implantable glucose sensor is an important objective in the management of diabetes mellitus. It could provide a more solid base for insulin administration and also be of use in the detection and therefore management of hypoglycaemia. Many investigators have researched the development of an implantable glucose sensor for continuous *in vivo* glucose monitoring. Consequently, there already is a wide range of different approaches and sensor designs available to study the feasibility of different implantation sites for the use of such a device [1–3].

An important problem encountered in most of these experiments was that the implanted sensors showed significant decay in sensitivity over the implantation period. This was not to be expected from their *in vitro* performance. This bioinstability hampers further clinical application of glucose sensors. Each time, it would require device replacement by surgical intervention.

A percutaneous device (PD), which provides permanent connection between the exterior and interior of the body, can perhaps solve the above-mentioned problem [4]. The PD can serve as a carrier for the glucose sensor and allow permanent access to various tissue compartments. Replacement of a sensor in case of impaired function will then be relatively easy without any surgery.

In view of the above-mentioned, it has to be noticed, that permanent access devices are still not without problems. Especially, when they are used to approach deeper tissue layers and/or body compartments. The catheter, as used in continuous ambulatory peritoneal dialysis (CAPD), is an example of such a device. This catheter penetrates both the skin and various muscle layers before entering the peritoneal cavity. Frequently observed complications are exit-site infections and peritonitis [5–7]. In addition, a correlation between the occurrence of peritonitis and exit-site infections is reported [8].

To solve these problems with access devices, we developed a new percutaneous implant. The main component of our design is a subcutaneous flange made of sintered titanium fibre mesh [9–11]. This mesh provides stability and allows the ingrowth of connective tissue. Until now, only histological information was available for our mesh implants when placed in a superficial subcutaneous position. On the other hand, for possible application as carrier of an implantable glucose sensor, such devices also have to be inserted in deeper tissue compartments without the risk of infection. Therefore, the purpose of this study was to evaluate the tissue reaction to a percutaneous access device based on the titanium fibre mesh principle in superficial and deeper body locations.

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2. Experimental procedure

2.1. Implants

Fig. 1 shows a schematic drawing of the implant used in this experiment. The implant consists of a silicone catheter provided with a titanium fibre mesh cuff with a fibre diameter of 50 μm, a volumetric porosity of 86% and a weight of 600 g m^{-2} at its peritoneal side. The titanium fibre mesh cuff is attached to the silicone tube with medical grade silicone glue. At the percutaneous side the catheter is connected to a percutaneous device consisting of a subcutaneous and a percutaneous component. The subcutaneous component comprises a sintered titanium fibre mesh sheet with a centrally disposed, rigid titanium holding element. The catheter is connected to the subcutaneous side of the holding element with Locktite 406 glue.

The percutaneous component consists of a polychloridetrifluoroethylene (KELF) connector. The percutaneous side of the catheter is notched and mechanically interlocked to the holding element.

2.2. Animals and surgical procedure

Seven healthy, adult (2–3 years of age), female Saane goats weighing approximately 60 kg were used in this experiment. In each goat, two implants were placed, one on the left and one on the right side of the spinal column. Before surgery, the implants were sterilized in an autoclave.

During the first surgical session, surgery was performed under general anaesthesia, induced by intravenous injection of penthobarbital 25 mg kg^{-1} and atropine 0.5 mg $animal^{-1}$. After oro-tracheal intubation, anaesthesia was maintained by ethrane 2–3% through a constant volume ventilator. The goat was situated on the operation table, lying on one side. Distal to the costal ridge, the skin was shaved, washed and disinfected with iodin. Parallel to the spinal column, an incision of about 4–5 cm was made through the skin. In between the skin and the cutaneous muscle layer, a pocket was carefully created towards the spinal column by blunt dissection with scissors. After haemostasis, the cutaneous muscle layer was perforated in the centre of the pocket.

At about 8 cm lateral to the first incision, a second incision was made through the skin and cutaneous muscle. After the subcutaneous fat layer was cleft, the various muscle layers were incised, parallel to the direction of the fibres. On reaching the peritoneum, this was opened and fixed with small musquito clamps. By means of a large clamp, a tunnel was created from the peritoneum, in between the various muscle layers, towards the central perforation of the cutaneous muscle layer at the pocket side. The large clamp was opened to fix the peritoneal side of the catheter, whereafter the catheter was guided through the tunnel. Subsequently, the titanium fibre mesh could be carefully placed inside the pocket and the incision was closed by means of vicryl 3–0 sutures. At the peritoneal side, the catheter was inserted in the abdominal cavity. The titanium fibre cuff was positioned in such a way that mechanical stresses at the subcutaneous fibre mesh were prevented. The peritoneum was closed around the catheter, whereafter the layers of muscle were approximated by means of vicryl sutures. Eventually the skin was closed. After an intervening healing period of four weeks, the head of the goat was fixed between two vertical bars. Through palpation, the subcutaneous fibre mesh together with the holding element was located. After local infiltration of the skin with lidocaine on top of the holding element, an incision of 1 cm was made. The silicone plug was removed and the percutaneous part of the catheter was adapted to the holding element. If necessary, the incision was closed with one or two sutures. The implants were left *in situ* for four months after placement of the percutaneous part. During this period the animals were stabled together in a separate room. Besides, the heads of the goats were fixed between two vertical bars to prevent the animals from manipulating the percutaneous devices. No additional covarage was applied on the exit sites. The goats were inspected once every two weeks. During these inspections the exit-sites of the percutaneous devices were carefully cleaned.

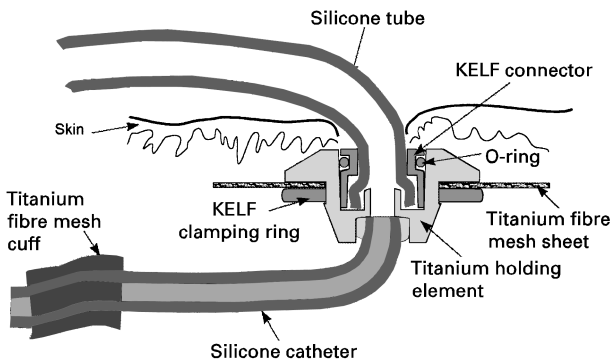

Figure 1 Graphical cross-section of the percutaneous access device used in the experiment.

2.3. Histologic procedure and evaluation

At the end of the experiment the goats were sacrificed using an overdose of Nembutal®. The implants with their surrounding tissues were excised immediately and fixated in 4% buffered formalin. After dehydration, excess tissue was removed and the samples were embedded in methyl methacrylate (MMA). The peritoneal part of the catheter was sectioned into two halves. Of each halve, sections were prepared in either longitudinal or transversal directions. The percutaneous part of the catheter was sectioned into four quarters. Histologic sections of approximately 10 μm were prepared using a sawing microtome, stained with methylene blue and basic fuchsin and investigated by light microscopy. The response of the soft-tissue to the implants was assessed by histologic and histomorphometric evaluation.

The tissue reaction to the peritoneal side of the catheter, the titanium fibre mesh cuff, was evaluated histomorphometrically. Therefore, an histologic grading scale was used as described previously by Jansen *et al.* (Table I) [12]. In summary, evaluation of the

TABLE I Histologic grading scale[a] for soft-tissue implants

	Response	Score
[a] Capsule		
Qualitatively	Capsule tissue is fibrous, mature, not dense, resembling connective or fat tissue in the non-injured regions	4
	Capsule tissue is fibrous but immature, showing fibroblasts and little collagen	3
	Capsule tissue is granulous and dense, containing both fibroblasts and many inflammatory cells	2
	Capsule consists of masses of inflammatory cells with little or no signs of connective tissue organization	1
	Cannot be evaluated because of infection or other factors not necessarily related to the material	0
Interstitium Qualitatively	Tissue in interstitium is fibrous, mature, not dense, resembling connective or fat tissue in the non-injured regions	4
	Tissue in interstitium shows blood vessels and young fibroblasts invading the spaces; few macrophages may be present	3
	Tissue in interstitium shows gaint cells and other inflammatory cells in abundance but connective tissue components in between	2
	Tissue in interstitium is dense and exclusively of inflammatory type	1
	Implant cannot be evaluated because of problems not related to the material tested	0

[a] Capsule Quantitatively, thickness rating:
1–4 fibroblasts 4
5–9 fibroblasts 3
10–30 fibroblasts 2
> 30 fibroblasts 1
Not applicable 0

capsule surrounding the implant was qualitative and semiquantitative, whereas evaluation of the interstitial tissue inside the fibre mesh was only quantitative. The semiquantitative classification consisted of a capsule thickness measurement based on the observed number of fibroblasts. The qualitative rating of the capsule and interstitium consisted of rating the tissue morphology (fibrous tissue, maturity, presence of connective tissue or fat tissue) and cellularity (presence of fibroblasts, macrophages, giant cells and other inflammatory cells).

To evaluate the tissue reaction to the percutaneous part of the catheter, sections of all four of the quadrants obtained were examined. Histological analysis consisted of evaluation of the epithelial attachment to the implant and the presence of an inflammatory reaction in the dermal connective tissue. Histomorphometrically, we measured the epithelial downgrowth and rated the quality of the tissue inside the titanium fibre mesh.

3. Results

3.1. Macroscopic clinical findings

None of the goats died during the experiment and the animals appeared to be in good health throughout the observation period. All the implants showed good healing with the surrounding soft-tissue. Around three implants, clinical signs of a mild inflammatory reaction were present. The implants showed various degrees of epithelial migration.

An important observation at the end of the implantation period was that in all but one of the implants the silicone catheter was disconnected from the subcutaneous part of the titanium holding element.

3.2. Microscopic and histomorphometric findings

The tissue reaction to the implants numbered 12 left and 18 left (Table II) could not be evaluated because of technical problems during the sectioning procedure. Furthermore, the interstitium quality of the implants 14 left and 15 right could not be determined because of poor quality of the sections obtained.

Light microscopic evaluation of the percutaneous part of the implants and their surrounding tissue showed various degrees of epithelial downgrowth, usually with the formation of a sinus tract. The sinus was filled with keratin. At the bottom of the sinus, the epidermis was attached to the implant surface (Fig. 2). In several cases epithelial downgrowth proceeded, resulting in exposure of the fibre mesh. The pores in the mesh were filled with connective tissue and blood vessels (Fig. 3).

Examination of the response to the tissue of the titanium fibre mesh cuff at the peritoneal side of the catheter showed that the tissue response was relatively uniform. All mesh implants were surrounded by a relatively thin fibrous capsule. The capsule contained fibrocytes, collagen and blood vessels and was commonly free from inflammatory cells (Fig. 4). Inside the fibre mesh the pores were filled with fibrous tissue containing blood vessels. Small numbers of lymphocytes, macrophages and foreign-body giant cells were present. Only occasionally, accumulations of inflammatory cells were seen (Fig. 5).

Furthermore, histological examination of the peritoneal cuff revealed that sometimes part of the fibre mesh was filled with silicone glue (Fig. 6). In a few sections the formation of a very dense fibrous capsule was observed between the silicone glue surrounding the catheter and the fibre mesh cuff.

The results of the histomorphometric evaluation of both the percutaneous and peritoneal part of the catheter are shown in Table II. The following

TABLE II Survey of histologic and histomorphometric findings

Goat	12	13	14	15	16	17	18	Mean ± SD
Percutaneous part								
Epithelial attachment								
Left implant	+	−	−	+	+	+	+	
Right implant	+	+	+	+	+	+	+	
Epidermal downgrowth (mm)								
Left implant	4.5	6.1	8.9	4.7	No	No	3.3	
Right implant	3.6	2.7	4.1	3.9	3.1	2.4	4.1	3.7 ± 2.2
Subepithelial inflammatory reaction								
Left implant	−	+	+	−	+	−	−	
Right implant	+	−	−	−	−	+	+	
Interstitium quality (ordinal score)								
Left implant	3	3		3	2	3	3	
Right implant	2	2	3		3	2	2	2.58 ± 0.51
Peritoneal part								
Capsule thickness (ordinal score)								
Left implant		2	2	3	2	3		
Right implant	2	2	3	3	2	2	3	2.29 ± 0.32
Capsule quality (ordinal score)								
Left implant		3	3	3	3	3		
Right implant	3	3	3	3	3	3	3	2.86 ± 0.19
Interstitium quality (ordinal score)								
Left implant		3	3	3	3	2		
Right implant	3	3	3	3	3	3	2	2.69 ± 0.33

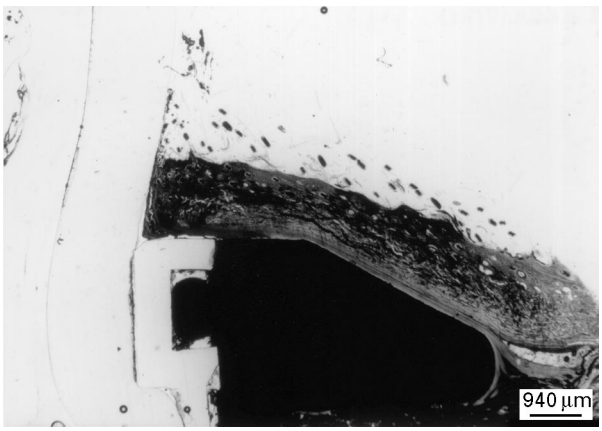

Figure 2 A histological cross-section of the percutaneous implant without clinical evidence of skin retraction. The percutaneous part of the device and the skin are shown.

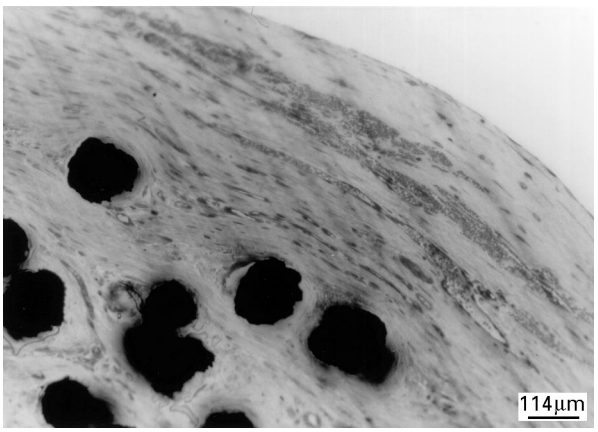

Figure 4 Histological section of the fibrous capsule surrounding the titanium fibre mesh cuff.

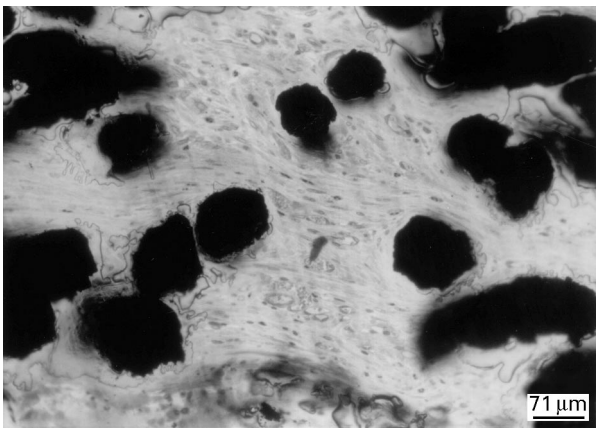

Figure 3 Histological section showing the mesh porosity of the titanium fibre mesh sheet filled with immature connective tissue and small blood vessels.

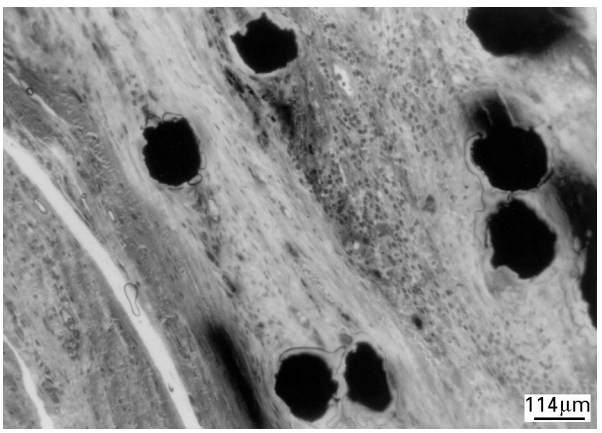

Figure 5 Histological section showing an occasional accumulation of inflammatory cells within the fibre mesh.

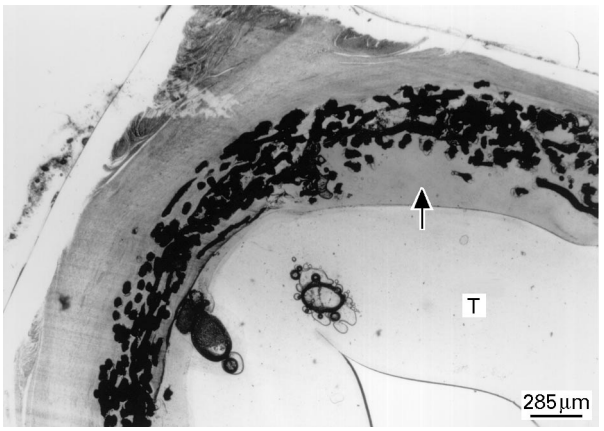

Figure 6 Histological section showing part of the fibre mesh porosity filled with the silicone glue (arrow) used to attach the fibre mesh cuff to the silicone tube (T).

observations were made

1. Epithelial attachment formation occurred with most percutaneous devices, except for the implants in goats 13 and 14.
2. The degree of epidermal migration varied – in two implants epidermal migration resulted in exposure of the holding element; around these implants also no epithelial attachment was observed.
3. In six implants a very mild subepithelial inflammatory reaction was observed. No relationship was observed between the existence of a subepithelial inflammatory reaction and the degree of epidermal migration.
4. The subcutaneous titanium fibre mesh sheets were all filled with immature connective tissue. In the interstium of five fibre meshes besides connective tissue, giant cells and other inflammatory cells were seen. Again, no clear relationship between the occurrence of inflammatory cells, subepithelial reaction and epithelial downgrowth could be observed.
5. All peritoneal titanium fibre mesh cuffs were surrounded by a relatively thin, connective tissue capsule.
6. In most cases, the peritoneal titanium fibre mesh cuff was filled with immature connective tissue. Only in implants 17 left and 18 right a mild inflammatory reaction was seen inside the mesh surrounding the dialysis catheter.

4. Discussion

The purpose of this investigation was to study the performance of a percutaneous access device applicable as a glucose sensor carrier, using titanium fibre mesh for soft-tissue anchorage. The access devices were implanted in the peritoneal cavities of goats. Permanent peritoneal access was created by connection of the catheter to a percutaneous device lateral of the spinal column using a two-stage implantation procedure. The tissue reaction to the percutaneous and peritoneal side of the device was evaluated histologically and histomorphometrically after four months of implantation.

In general, a good tissue reaction to the percutaneous device was observed. In most implants there was only limited epidermal migration. In two implants, epidermal migration proceeded with exposure of the titanium holding element. In six implants, a very mild inflammatory reaction of the dermal connective tissue was observed. In three implants, clinical signs of mild inflammation were present. In the other implants, the subepithelial inflammatory reaction did not result in clinical signs. These results are in contrast with the findings of our previous experiments with percutaneous devices [9, 10]. In these investigations the tissue reaction to soft-tissue anchored percutaneous implants in rabbits was evaluated. No inflammatory reaction of the dermal connective tissue surrounding the implants was observed. An explanation for these contradictory results is the presence of mechanical irritation in the currently used goat model. Despite fixation of their heads between bars, the goats were able to rub the implantation sites against each other. This mechanical irritation will eventually influence the soft-tissue response to the penetrating percutaneous part of the implant. For future experiments solutions have to be found for this problem.

In addition, we have to emphasize that sinus tract development around the device creates an access for bacteria into the cutaneous tissue and can result in infection. Further investigations are needed to assess this relationship and to study the influence of contributing factors, such as surgical procedure, duration of implantation, implant design, post-operative care, host defense mechanisms, and mechanical stresses on the device.

Histological evaluation also revealed that all subcutaneous titanium fibre mesh sheets were filled with immature connective tissue, mostly free of inflammation. This is in agreement with the results of all our earlier investigations showing that sintered titanium fibre-web structures induce the ingrowth of fibrous tissue and effectively stabilize percutaneous devices localized in subcutaneous soft-tissue [9–13].

This study also proved that the titanium cuff attached to the peritoneal part of the catheter showed sufficient ingrowth of vascularized connective tissue, generally free of inflammation. This indicates that titanium fibre mesh structures also promote the ingrowth of connective tissue in deeper soft-tissue layers. This is a beneficial effect in comparison with Dacron® velour cuffs, that only show limited ingrowth of connective tissue [14]. It can be supposed that this ingrowth will contribute to the prevention of infection.

Although the results of this study confirm the biocompatible behaviour of titanium fibre mesh structures, still more research is needed to investigate the relationship between the amount and maturation of ingrowing connective tissue and the degree of subcutaneous anchorage. Changes in porosity or diameter of the fibres of the titanium fibre mesh can, for example, influence ingrowth behaviour.

Also surface topography of the implant materials used may influence the response of the host [15]. Wan

et al. studied cell behaviour on the surfaces of multifilament materials [16]. They were able to show a significant orientational behaviour of cells on fibre surfaces, independent of the bulk chemistry of the fibres. This phenomenon shows similarities with the general influence of surface microtopography on cell behaviour, known as contact guidance [17–19]. Besides orientation, it is suggested that microtopography of a surface can influence fibrous capsule formation around implants [15]. At the moment no data are available to support this hypothesis.

Finally, some critical remarks have to be made about the design and manufacturing of the percutaneous devices used. First, in all but one implant the silicone catheter was found to be disconnected from the titanium holding element after four months of implantation. Probably this was due to a combination of the mechanical stresses, induced by the rubbing of the goats against each other and dissolution of the cyanoacrylate glue in the tissue environment. This problem can be overcome by the design of a mechanical interlock between the tube and the holding element. Further, we have to notice that this problem occurred despite extensive preclinical mechanical testing of our devices. Therefore, this shows again that other, more appropriate, methods have to be developed for the *in vitro* screening of new devices. Perhaps, the use of simulated body conditions offers a solution [20, 21].

Another problem observed in this study was that part of the fibre mesh cuff at the peritoneal side of the catheter was filled with the glue used to attach the cuff to the silicon tube. This inhibited the ingrowth of fibrous tissue into some parts of the cuff and could have influenced its performance. Although, similar observations have been made in the Dacron® cuffs on commercially available dialysis catheters, this penetration of glue inside the mesh material has to be a point of attention in future implant designs.

5. Conclusions

These experiments have demonstrated that titanium fibre mesh structures can be used for soft-tissue anchorage of a percutaneous access device. A sufficient ingrowth of connective tissue was obtained in superficial as well as in deeper soft-tissue layers. Considering these observations, such access devices can have an application as carriers of an implantable glucose sensor for continuous glucose monitoring.

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References

1. D. A. GOUGH and J. C. ARMOUR, *Diabetes* **44** (1995) 1005.
2. E. F. PFEIFFER, *Horm. Metab. Res. Suppl.* **24** (1990) 154.
3. G. S. WILSON, Y. ZHANG, G. REACH, D. MOATTI-SIRAT, V. POITOUT, D. R. THEVENOT, F. LEMONNIER and J.-C. KLEIN, *Clin. Chem.* **38** (1992) 1613.
4. A. F. VON RECUM and J. B. PARK, *CRC Crit. Rev. Bioeng.* **5** (1981) 37.
5. J. W. MONCRIEF and R. P. POPOVICH, in "*Peritoneal dialysis*", 3*rd Edn*, edited by K. D. Nolph (Kluwer Academic, Dordrecht, The Netherlands, 1988) p. 152.
6. B. PIRAINO, J. BERNARDINI and M. I. SORKIN, *Amer. J. Kidney Dis.* **8** (1986) 436.
7. A. SCALAMOGNA, C. CASTELNOVO and A. DE VECCHI, *ibid.* **18** (1991) 674.
8. Y. C. G. J. PAQUAY, J. A. JANSEN, R. J. A. GORIS and A. J. HOITSMA, *J. Invest. Surg.* **9** (1996) 81.
9. J. A. JANSEN, J. P. C. M. VAN DER WAERDEN and K. DE GROOT, *J. Biomed. Mater. Res.* **25** (1991) 1535.
10. J. A. JANSEN, Y. C. G. J. PAQUAY and J. P. C. M. VAN DER WAERDEN, *ibid.* **28** (1994) 1047.
11. Y. C. G. J. PAQUAY, J. E. DE RUIJTER, J. P. C. M. VAN DER WAERDEN and J. A. JANSEN, *ibid.* **28** (1994) 1321.
12. J. A. JANSEN, W. J. A. DHERT and J. P. C. M. VAN DER WAERDEN, *J. Invest. Surg.* **7** (1992) 123.
13. Y. C. G. J. PAQUAY, J. E. DE RUIJTER, J. P. C. M. VAN DER WAERDEN and J. A. JANSEN, *Amer. Soc. Artif. Intern. Organs* **42** (1996) 961.
14. *Idem*, *Biomaterials* **17** (1996) 1251.
15. A. F. VON RECUM, C. E. SHANNON, C. E. CANNON, K. J. LONG, T. G. VAN KOOTEN and J. MEYLE, *Tissue Engng* **2** (1996) 241.
16. H. WAN, R. L. WILLIAMS, P. J. DOHERTY and D. F. WILLIAMS, *J. Mater. Sci. Mater. Med.* **8** (1997) 45.
17. E. T. DEN BRABER, J. E. DE RUIJTER, H. T. J. SMITS, L. A. GINSEL, A. F. VON RECUM and J. A. JANSEN, *J. Biomed. Mater. Res.* **29** (1995) 511.
18. A. S. G. CURTIS and P. CLARK, *Crit. Rev. Biocompat.* **5** (1990) 343.
19. D. M. BRUNETTE, in "*Surface characterization of biomaterials*", edited by B. D. Ratner (Elsevier, Amsterdam, The Netherlands, 1988) p. 203.
20. J. C. G. WOLKE, J. P. C. M. VAN DER WAERDEN, K. DE GROOT and J. A. JANSEN, *Biomaterials* **18** (1997) 483.
21. P. LI, C. OHTSUKI, T. KOKUBO, K. NAKANISHI, N. SOGA, T. NAKAMURA and T. YAMAMURO, *J. Appl. Biom.* **4** (1993) 221.

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