Short-term *in vitro* and *in vivo* biocompatibility of a biodegradable polyurethane foam based on 1,4-butanediisocyanate

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In this study short-term *in vitro* and *in vivo* biocompatibility apects of a biodegradable polyurethane (PU) foam were evaluated. The PU consists of hard urethane segments and amorphous soft segments based on a copolyester of dl-lactide and ε-caprolactone. The urethane segments are of uniform length and synthesized with 1,4-butanediisocyanate. The foam has good mechanical properties and will be used for tissue regeneration applications. Degradation tests were carried out in a buffer solution for twelve weeks. Cytotoxicity was determined using extract and direct contact test methods with incubation periods varying form 24 to 72 h. The foam was implanted subcutaneously for one, four and twelve weeks and the tissue response to the material was histologically evaluated.

In vitro, the mass loss was 3.4% after twelve weeks. In the cytotoxicity tests the PU caused no abnormal growth behaviour, nor morphological changes or inhibition in metabolic activity. The *in vivo* studies showed no toxic tissue response to the PU. Connective tissue ingrowth, accompanied by vascular ingrowth was complete at twelve weeks. *In vivo* degradation had started within four to twelve weeks.

In conclusion, the PU shows a good *in vitro* and *in vivo* biocompatibility in these short-term experiments.

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1. Introduction

Biodegradable materials have been applied in medical practice for over thirty years. In the past, these materials were mainly used in sutures and osteosynthesis materials [1–3]. These devices have a non-permanent function. If made of a biodegradable material, their removal from the body after they have performed their function is not necessary, which is considered a major advantage over non-degradable materials. This advantage also accounts for the more recent applications of biodegradable materials, such as scaffolds for tissue regeneration and engineering [4].

Polylactides, polyglycolides and combinations of these materials are the most widely applied biodegradable materials. It has been shown that the short-term biocompatibility of these materials is acceptable [5, 6]. However, during degradation complications may occur. In case of crystalline polylactides, like Poly-L-Lactide (PLLA), the degradation process is slow [7] and remaining particles can cause a chronic foreign body reaction [8, 9]. If degradation occurs too fast, complications can be initiated by the quick drop in pH and the release of polymer particles that may overwhelm the cleaning capacity of the body [10, 11].

In general, biodegradable polymers have mechanical properties that are significantly less than permanent materials [12, 13]. The mechanical properties of crystalline polylactides are relatively good but the slow degradation and the problems with long-term biocompatibility limit their applications [12].

An ideal biodegradable material would have appropriate mechanical properties combined with a good biocompatibility on the short term as well as during degradation. Until recently, polyurethanes (PUs) were not considered to meet these criteria, because they were produced using aromatic diisocyanates [14].

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Degradation of these PUs leads to the release of potential carcinogenic components.

New biodegradable PUs developed by Spaans *et al.* [15], are synthesized with an aliphatic diisocyanate (1,4-butanediisocyanate). Hydrolysis of the urethane bond leads to the formation of butanediamine. This substance is a normal constituent of all mammalian cells and plays a role in the regulation of cell growth. The catabolites are excreted in urine [16].

The purpose of this study was to evaluate the short-term biocompatibility and degradation of the new biodegradable PU *in vitro* and *in vivo*. The material was tested as a highly porous foam, as we are planning to use it for guided tissue regeneration applications.

2. Materials and methods

2.1. Materials

A random prepolymer of 50% DL(50/50)-lactide and 50% ε-caprolactone (Mn = 2000) was obtained by ring opening polymerization of the monomers with the required amount of 1,4-butanediol (BDO) initiator at 130 °C for 120 h using stannous octoate as catalyst (0.03 w/w%). The resulting macrodiol was end-capped with a six-fold excess of 1,4-butanediisocyanate (BDI) at 60 °C and the excess was distilled off. Chain extension was performed in 40 w/w% 1,4-dioxane solution at 70 °C using a BDO-BDI-BDO urethane chain extender. The resulting PU consisted for 22.5 w/w% of hard urethane segments and for 77.5 w/w% of amorphous polyester soft segments.

The highly viscous polyurethane solution was diluted with 1,4-dioxane to a concentration of 4 w/w%. After addition of 7.5 w/w% water the solution was poured into a mould. The solution was cooled down to −18 °C followed by freeze drying (1 mbar) to remove the water and dioxane crystals. The porosity of the PU foams was 94 v/v% and this was calculated by determination of weight ($d = 1.1$ g/cm^3) and volume. SEM studies of the foams showed a pore size of 100–300 μm with interconnective pores of 10–30 μm (Fig. 1).

In all tests, PU foams were compared to foams made of high molecular weight poly (dl-lactide-co-ε-caprolactone), a copolymer (CP) of similar components as the soft segments of the PU. This CP was obtained by ring opening polymerization of the required monomers at 110 °C for 11 days using stannous octoate as catalyst. Foams of the CP were prepared using the same method as for the PU foams.

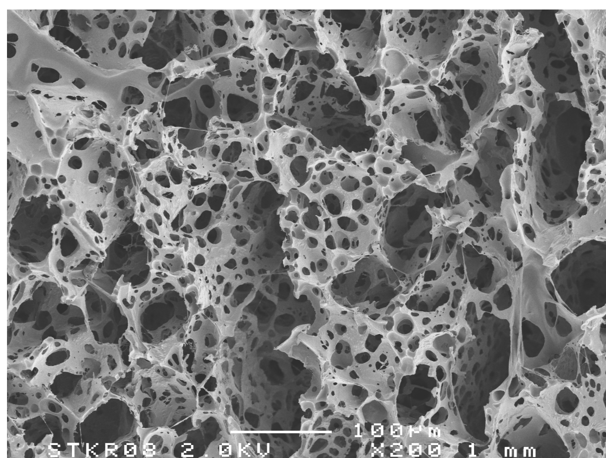


Figure 1 Porous structure of the PU foam, pictured with scanning electron microscopy.

Commercially available ultra high molecular weight polyethylene (UHMW-PE, obtained from Goodfellow, UK) served as a non-degradable control material. Both control materials have been shown to have a good short-term biocompatibility [17, 18]. All materials were sterilized with ethylene oxide.

2.2. *In vitro* degradation study

Cylindrical PU and CP foams were degraded at a temperature of 37 °C in separate tubes containing 30 ml sterile Sörensen buffer solution (pH 7.4). The mean (±standard deviation) weight was 180 ± 5.6 mg of PU foams and 167 ± 11.6 mg of the CP foams. The PU samples had a length of 3.55 ± 0.10 cm, with a diameter of 0.97 ± 0.03 cm. The CP samples had a length of 2.64 ± 0.13 cm and a diameter of 0.75 ± 0.03 cm.

According to ISO standard 15814, three samples were taken for each period and pH was measured at fixed time intervals. If necessary, the pH was adjusted to 7.4 ± 0.3 with 1.0 N NaOH.

After periods varying from 1 day to 12 weeks the degrading foams were filtered over a preweighed 0.45 μm filter (Durapore membrane filter, Milipore). The degradation products remaining on the filter were rinsed with distilled water to remove residual buffer salts. After freeze-drying, the filters were weighed to determine the mass of the remaining foam.

2.3. Cytotoxicity tests

Extraction and direct contact tests were used to evaluate the cytotoxicity of the non-degraded materials [19, 20]. The tests were based on ISO 10993-5 standards. Mouse fibroblasts (L929) were cultured in RPMI-1640 medium, supplemented with 10% fetal calf serum, 1% penicillin streptomycin and 1% L-Glutamin (all ingredients obtained from Gibco). Extracts were prepared in the same culture medium. All cytotoxicity tests were evaluated at 24, 48 and 72 h. Latex rubber served as a positive control. A blank control was included to evaluate the influence of the procedure. After the incubation period, confluency of the cell monolayers was compared to the controls and the cells were evaluated for lysis, morphology, and cell growth. In both extract and direct contact tests the cells were counted. An MTT procedure was performed to test the metabolic activity of the cells exposed to extracts [19]. The optical density of the MTT conversion product was measured with a microplate reader (BioRad, model 3550-UV) at 595 nm. The MTT test was performed in sixfold, all other tests were performed in triplicate.

A one way analysis of variance (ANOVA) was used to compare the results of the PU to the controls (significance level 0.05). In case of a statistically significant result, a multiple comparison test according to Tukey was performed [21].

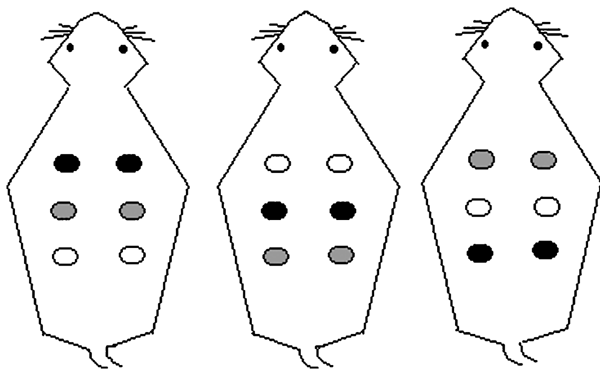


Figure 2 PU, CP and PE samples implanted on different locations in the back of the rats.

2.4. Subcutaneous implantation study

Nine six-week-old male Wistar rats were anaesthesized with halothane. Discs measuring 10 mm in diameter and 1 mm in thickness were implanted in subcutaneous pockets on the back. Two discs of each biomaterial (PU, CP, PE) were implanted in each rat (Fig. 2). Per time interval, three rats were used to evaluate a total of six discs of each material. To exclude influences caused by the location of implantation the rats had varying sample configurations.

The rats were housed according to the Dutch national code of practice for animal welfare and had free access to standard food and water. At 1, 4 and 12 weeks the animals were sacrificed by cervical dislocation. The skin of the back was cut and the samples were located and inspected macroscopically. The samples were

TABLE I Scoring parameters of the tissue response to the PU and control materials

Parameter	Score
1. Necrosis	yes/no
2. Thickness of fibrous capsule	number of cell layers
3. *Number of inflammatory cells*	
3a. Polymorphonuclear cells[1]	0–4
3b. Macrophages[1]	0–4
3c. Foreign Body Giant Cells[1]	0–4
4. *Tissue ingrowth into the foam*	
4a. Ingrowth of connective tissue[2]	0–4
4b Ingrowth of tissue accompanied by vascularisation	yes/no
5. *Foam*	
5a. Signs of degradation[3]	0–4
5b. Cells with phagocytic activity[1] (foamy cells, large cytoplasm)	0–4

[1]Numbers of inflammatory cells or cells with phagocytic activity range from hardly any cell present (0) to a high number of cells (4).
[2]The score of tissue ingrowth varies from no ingrowth (0) to infiltration of tissue into the whole area of the foam (4).
[3]Loss of foamy structure or diffuse staining of the foam were defined as signs of degradation. The score varies from no signs of degradation (0) to degradation in the centre of the foam (4).

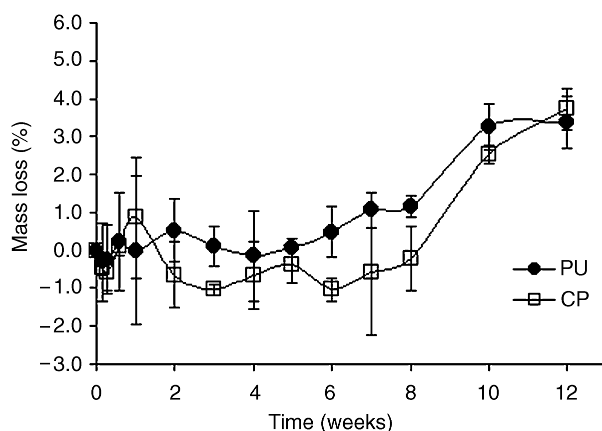
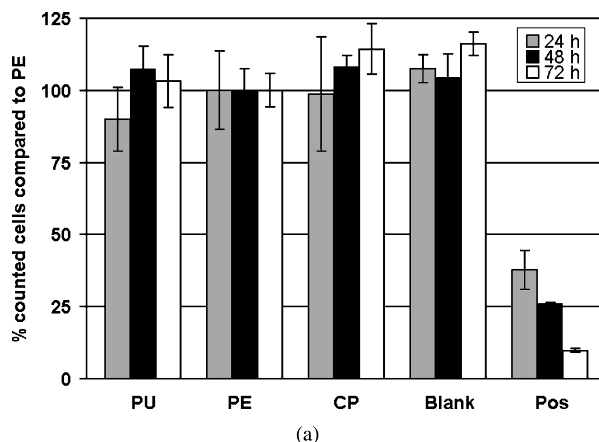
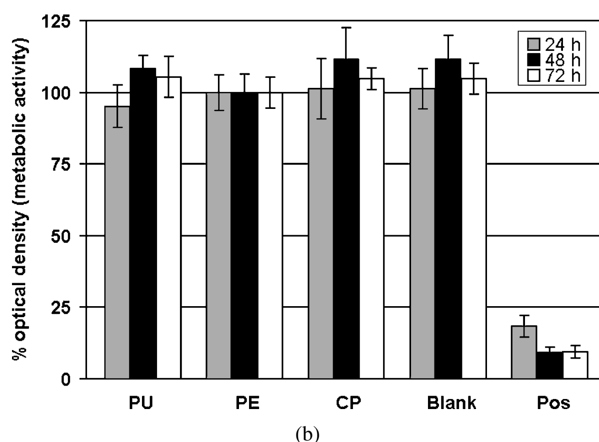


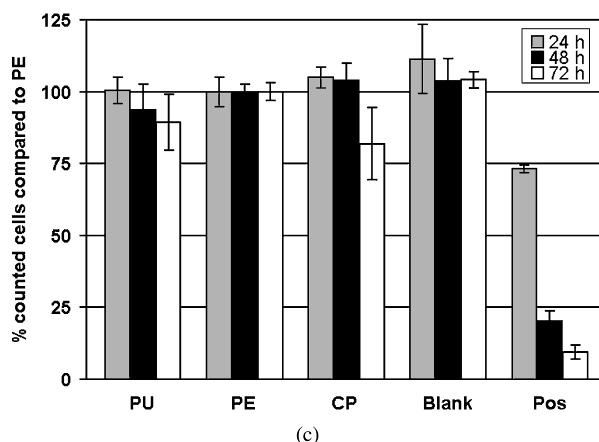
Figure 3 In vitro mass loss (%) of PU and CP at 37 °C in Sörensen buffer solution at pH 7.4 (0–12 weeks).



(a)



(b)



(c)

Figure 4 Results of the cell count of the extract test (4a), MTT procedure (4b), and the cell count of the direct contact test (4c) after 24, 48, and 72 h of incubation. The optical density of the samples (4b) is a measure for the metabolic activity of the cells. The error bars indicate the standard deviation ($n = 3$ in Fig. 3(a) and (c), $n = 6$ in Fig. 4(b)). (Pos = positive control).

then explanted, fixed in 4% phosphate buffered formalin, and embedded in GMA. They were cut perpendicular into two series of sections with a thickness of 2 μm and were stained with Toluidin Blue and Toluidin Blue/Basic Fuchsin. The tissue response was evaluated using a semi-quantitative scoring method (Table I). A one way analysis of variance (ANOVA) was used to compare the number of cell layers in the fibrous capsule to the controls (significance level 0.05).

3. Results

3.1. *In vitro* degradation

At 12 weeks the average mass loss of the PU foam was 3.4%. The CP foams lost an average of 3.7% of their mass. Fig. 3 shows the results of all measurements.

3.2. Cytotoxicity tests

The results of the cell counts and metabolic activity are shown in Figs. 4(a)–(c). In the graphs the results of the tests were compared to the results of the non-degradable negative control (PE), which was fixed at 100%.

The cell count of the extraction test is plotted in-Fig. 4(a). In this experiment the cells exposed to PU showed no morphological abnormalities after any incubation period. The number of lysed cells did not differ from the solid negative control material (PE). This also applied to the cells exposed to CP. Most cells exposed to the positive control lost attachment from the culture wells and morphology and growth were poor. The metabolic activity of the fibroblasts is shown in Fig. 4(b).

In Fig. 4(c) the cell count after 24, 48, and 72 h of direct exposure to the test material is presented. Again, no morphological abnormalities were observed in the cells exposed to PU, CP and PE. Almost all cells exposed to the positive control were in very poor condition. Statistical analysis showed that the metabolic activity and cell counts of the cells exposed to PU did not differ significantly from the PE, CP and blank samples in any experiment (p-values ranging from 0.06 to 0.56). The scores of the positive control were significantly lower than the other scores ($p \ll 0.05$ in all cases).

3.3. Subcutaneous implantation

The postoperative period and the wound healing were uneventful in all rats. On explantation there were no signs of infection or necrosis at one, four and twelve weeks. After one week the samples were macroscopically embedded within the subcutaneous tissue in a thin fibrous layer. This fibrous layer was thicker and denser after four and twelve weeks. After one week a haematoma was observed around two PE samples near the hind legs of the rat. For this and other reasons an occasional sample could not be evaluated histologically.

The averaged histological results are summarized in Table II. No necrosis was observed in any sample at any time interval. The number of cell layers in the fibrous capsule around the PU did not differ significantly from the controls after one and four weeks. At twelve weeks the PE capsule consisted of a significantly higher number of cell layers ($p = 0.006$).

At one week, a slight ingrowth of tissue was observed into the PU and CP foams (Fig. 5(a)–(b)). After four weeks this ingrowth had increased and at twelve weeks the tissue had infiltrated nearly the whole area of the foams (Fig. 5(c)–(f)). In all cases the tissue ingrowth was accompanied by vascularisation (Fig. 5(g)). In the infiltrated areas, both the PU and CP foams showed signs of degradation, i.e., loss of foamy structure and diffuse staining of the biomaterials.

No abundance of polymorphonuclear cells was seen in any sample. In contrast to PE, the PU and CP foam samples showed increasing numbers of macrophages and giant cells. We found no differences in the amount of giant cells and macrophages between the PU and CP foams at the different time intervals. After four weeks an occasional foamy macrophage was observed in the PU samples and after twelve weeks the number of foamy macrophages had increased to a 'moderate number' (Fig. 5(f)).

TABLE II Average results of histological evaluation after one, four and twelve weeks

	1 Week			4 Weeks			12 Weeks		
Sample	PU	CP	PE	PU	CP	PE	PU	CP	PE
Number of evaluated samples ($n = 6$)	6/6	6/6	4/6	6/6	5/6	6/6	5/6	5/6	6/6
1. Necrosis (y/n)	n	n	n	n	n	n	n	n	
2. Fibrous capsule (number of cell layers ± standard deviation)	5.8 ± 1.7	8.5 ± 6.7	14.3 ± 5.1	8.5 ± 4.3	7.2 ± 1.1	14.0 ± 8.1	5.8 ± 1.8	4.6 ± 1.5	15.5 ± 7.8
3a. PMN's (0–4)	0	0	0	0	0	0	0	0	0
3b. MP's (0–4)	1	1	0	2	2	0	2	2	0
3c. FBGC's (0–4)	1	1	0	1	1	0	2	2	0
4a. Tissue ingrowth (0–4)	1	1	*	3	2	*	4	4	*
4b. Vascularisation (y/n)	y	y	*	y	y	*	y	y	*
5a. Degradation (0–4)	1	1	*	3	4	*	4	4	*
5b. Cells with phagocytic activity (0–4)	0	0	*	1	0	*	2	0	*

See Table I for explanation of the scores. As PE is a non-porous, non-degradable material several items could not be evaluated (indicated by *)

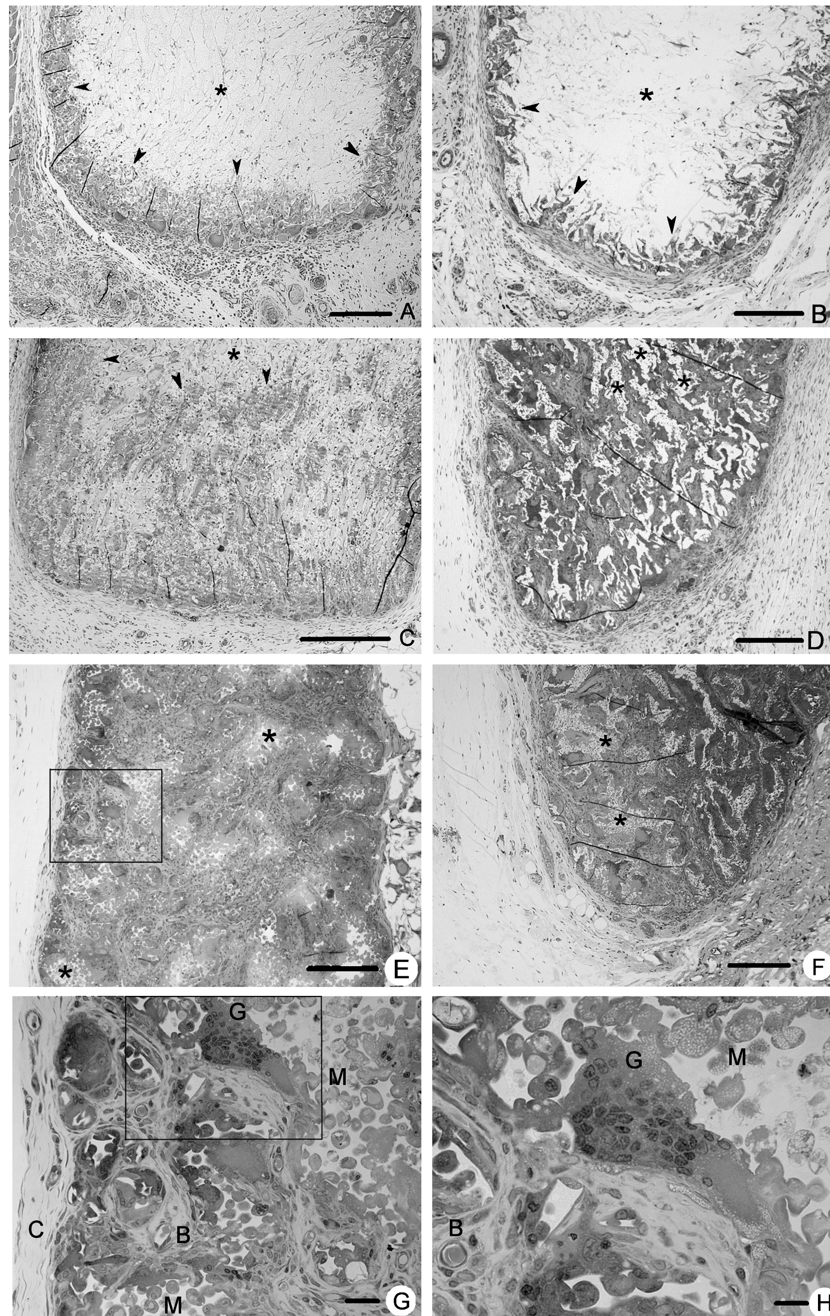


Figure 5 (a) and (b): PU (a) and CP (b) foam after one week of implantation. A rim of tissue has grown into the foams (arrow). The larger part of the original foams has not been reached by ingrowing tissue (asterisk). The surrounding tissues show no signs of acute inflammation. (Toluidin blue/basic fuchsin, bar = 200 μm). (c) and (d): PU (c) and Cp (d) foams after four weeks of implantation. The connective tissue has grown further into both foams. Small strands of tissue have almost reached the middle of the CP foam. The asterisks indicate areas that have not been reached by tissue. The PU foam shows a different pattern of ingrowth. The border of the tissue is indicated with arrows. (Toluidin blue/basic fuchsin, bar = 200 μm). (e) and (f): PU (e) and CP (f) foams after twelve weeks of implantation. The tissue has reached the centre of both foams. Only small areas have not been reached by fibrous tissue (asterisks). (Toluidin blue/ basic fuchsin, bar = 200 μm). (g): Detail of Fig. 5(e). Connective tissue accompanied by blood vessels (B) grows into the foam. In this sample the fibrous capsule (C) has a thickness of 7 layers of cells. Foreign body giant cells (G) can be found near the tissue-foam margin. (Toluidin blue/ basic fuchsin, bar = 50 μm). (h): Detail of Fig. 5(g). The same foreign body giant cell is indicated (G). Foamy macrophages (M) and blood vessels (B) can be observed in detail. (Toluidin blue/basic fuchsin, bar = 25 μm).

4. Discussion and conclusions

Biocompatibility is a major issue in the development of new biodegradable materials for guided tissue regeneration and tissue engineering. Recently, Agrawal emphasized the importance of the biocompatibility of polymeric scaffolds for tissue engineering purposes [22].

As problems with the biocompatibility may occur at several stages of degradation, the biocompatibility of new materials should be assessed at several stages. A non-degraded material and the potential presence of oligomers, solvents or other toxic components determine the short-term biocompatibility. During degradation, elements may be produced that endanger the medium-term biocompatibility. Finally, residual products from the material may cause problems in the long-term [23].

In this study we evaluated the short-term biocompatibility of a new biodegradable PU foam. The biocompatibility of the PU was screened *in vitro* by performing cytotoxicity screening tests. In the past, many

biodegradable materials have been tested and introduced for various medical applications. Surprisingly, in most cases the biocompatibility and application of these materials have been first tested *in vivo*. When complications in clinical use occurred, *in vitro* experiments were performed to clarify their cause [24]. In order to anticipate possible future complications, we have tested the biocompatibility of the PU foam *in vitro*, before performing *in vivo* experiments.

Proper control materials are essential to obtain reliable information on the biocompatibility of a new biomaterial. The biocompatibility of PE is beyond discussion. It has been used in many medical applications for many years and is often used as a control material in biocompatibility tests [25, 26]. Although all samples were of the same shape and size [27] in all tests, we did not regard the use of PE as the only control material to be sufficient. PE is both a hard nondegradable and non-porous material. These physical properties may cause mechanical irritation in the *in vivo* study. We observed some haematomas in the mobile area near the hind legs of the rats, which may have been caused by the PE damaging surrounding tissue and vessels.

Amorphous copolymers of DL-lactide and ε-caprolactone have been shown to have a good biocompatibility *in vivo* [18]. In addition, the copolymer foams are as flexible as the PU foam. Therefore, we considered this material to be a suitable degradable control material.

Various *in vitro* tests were performed to acquire reliable cytotoxicity data [28]. In all tests performed, neither the number of cells, nor the metabolic activity of the cells exposed to PU differed from the negative (PE and CP) and blank controls. Morphological abnormalities of cells exposed to PU were not observed. Therefore, we conclude that the non-degraded polyurethane foam shows a good biocompatibility *in vitro*.

The histological results from the *in vivo* study indicate a good short-term biocompatibility of the PU foam. There were no signs of acute inflammation or necrosis. The fibrous capsule formation around the PU foams was comparable to the CP foams and remained thin in comparison with PE. The fast ingrowth of connective tissue was accompanied by blood vessels. This is a promising indication for its biocompatibility as well.

The increasing numbers of macrophages and foreign body giant cells in the PU samples may raise questions as to the biocompatibility of the foam. These types of cells were also present in the CP samples, but were absent around the PE samples. In another study with CP these high numbers of macrophages were also observed [18]. This was determined as a transient response to the degrading material. At this moment we ascribe the high numbers of these cells to be a normal response during the first stages of the degradation process and not to a lack of biocompatibility.

In vitro, the mass loss of both the PU and CP foam was very low at twelve weeks and degradation seemed to have just started. However, mass loss is only one aspect of degradation. We assume that the polymer chain length decreases due to hydrolysis during this period, but has as yet not resulted in a substantial loss of total mass.

As to the *in vivo* studies, the first signs of degradation of the rim of the foams can already be observed at one week. In Figs. 5(c)–(f) it can be seen that the pattern of ingrowth into the PU foam is different from the ingrowth into the CP foams. This leads to temporary differences in tissue penetration and signs of degradation at four weeks. However, at twelve weeks nearly the whole area of both foams is penetrated by tissue and cells, accompanied by signs of degradation in the whole area of both foams (Table II).

The most striking difference observed between the PU and CP foams was the presence of large foamy macrophages in the PU samples (Figs. 5(f)–(g), Table II). The presence of the urethane segments might account for this finding. Hydrolysis of CP and the soft segments of the PU is well reported [29] and we assume that this occurs more easily than the hydrolysis of the urethane bonds. As to the PU foams, macrophages will commence with phagocytosis of the small particles of the PU after the decrease of polymer chain length by hydrolysis of the soft segments. It remains to be seen whether these macrophages are capable of further degradation of the enclosed PU particles.

We conclude that in both the *in vivo* and *in vitro* study reveal a promising short-term biocompatibility of the PU foam . As has been stated by several authors, short-term biocompatibility is only one important property of a biodegradable material [4, 22]. Future research of the PU foam will involve long term studies focused on the biocompatibility, the degradation, and application of this material.

Acknowledgments

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References

1. D. E. CUTRIGHT, E. E. HUNSUCK and J. D. BEASLY, *J. Oral. Surg.* **29** (1971) 393.
2. L. GETTER, D. E. CUTRIGHT, S. N. BHASKAR and J. K. AUGSBURG, *ibid.* **30** (1972) 344.
3. R. R. M. BOS, G. BOERING, F. R. ROZEMA and J. W. LEENSLAG, *J. Oral Maxillofac. Surg.* **45** (1987) 751.
4. D. W. HUTMACHER, *Biomaterials* **21** (2000) 2529.
5. S. J. GOURLAY, R. M. RICE, A. F. HEGYELI, C. W. R. WADE, J. G. DILLON, H. JAFFE and R. K. KULKARNI, *J. Biomed. Mater. Res.* **12** (1978) 219.
6. J. M. BRADY, D. E. CUTRIGHT, R. A. MILLER and G. C. BATTISTONE, *ibid.* **7** (1973) 155.
7. O. LAITINEN, H. PIHLAJAMAKI, A. SUKURA and O. BÖSTMAN, *ibid.* **61** (2002) 33.
8. J. E. BERGSMA, W. C. DE BRUIJN, F. R. ROZEMA, R. R. M. BOS and G. BOERING, *Biomaterials* **16** (1995) 25.
9. O. BÖSTMAN, H. K. PIHLAJAMÄKI, E. K. PARTIO and P. U. ROKKANEN, *Clin. Orthop.* **320** (1995) 101.
10. M. S. TAYLOR, A. U. DANIELS, K. P. ANDRIANO and J. HELLER, *J. Appl. Biomater.* **5** (1994) 151.
11. O. BÖSTMAN, E. HIRVENSALO, J. MÄKINEN and P. ROKKANEN, *J. Bone Joint Surg. Br.* **72b** (1990) 592.
12. L. E. CLAES, *Clin. Mater.* **10** (1992) 41.

13. W. S. PIETRZAK, D. SARVER and M. VERSTYNEN, *Bone* **19** (1996) 109.
14. J. H. DE GROOT, R. DE VRIJER, A. J. PENNINGS, J. KLOMPMAKER, R. P. H. VETH and H. W. B. JANSEN, *Biomaterials* **17** (1996) 163.
15. C. J. SPAANS, "Biomedical Polyurethanes Based on, 4-butanediisocyanate: An Exploratory Study," Thesis, State University of Groningen, The Netherlands, 2000.
16. V. W. RODWELL, in "Harper's Biochemistry" (Appleton & Lange, New York, 2000) p. 347.
17. W. C. RICHARDSON, J. J. KLAWITTER, B. W. SAUER, J. R. PRUITT and S. F. HULBERT, *J. Biomed. Mater. Res.* **6** (1975) 73.
18. W. F. A. DEN DUNNEN, P. H. ROBINSON, R. VAN WESSEL, A. J. PENNINGS, M. B. M. VAN LEEUWEN and J. M. SCHAKENRAAD, *ibid*. **36** (1997) 337.
19. A. DEKKER, C. PANFIL, M. VALDOR, G. PENNARTZ, H. RICHTER, CH. MITTERMAYER and C. J. KIRKPATRICK, *Cells Mater.* **4** (1994) 101.
20. S. A. ROSENBLUTH, G. R. WEDDINGTON, W. L. GUESS and J. AUTIAN, *J. Pharm. Sci.* **54** (1965) 156.
21. D. G. KLEINBAUM, L. L. KUPPER, K. E. MULLER and A. NIZAM, in "Applied Regression Analysis and Multivariable Methods" (Duxbury Press, Pacific Grove, 1998) p. 447.
22. C. M. AGRAWAL and R. B. RAY, *J. Biomed. Mater. Res.* **55** (2001) 141.
23. H. PISTNER, R. GUTWALD, R. ORDNUNG, J. REUTHER and J. MÜHLUNG, *Biomaterials* **14** (1993) 671.
24. F. W. CORDEWENER and J. P. SCHMITZ, *Tissue Eng.* **6** (2000) 413.
25. W. C. RICHARDSON, J. J. KLAWITTER, B. W. SAUER, J. R. PRUITT and S. F. HULBERT, *J. Biomed. Mater. Res*. **6** (1975) 73.
26. B. D. RATNER, A. F. HOFFMAN, F. J. SCHOEN and J. E. LEMONS, in "Biomaterials Science: An Introduction to Materials in Medicine" (Academic Press, San Diego, 1996).
27. B. F. MATLAGA, L. P. YASENCHAK and T. N. SALTHOUSE, *J. Biomed. Mater. Res.* **10** (1976) 391.
28. C. J. KIRKPATRICK and A. DEKKER, *Adv. Biomaterials* **10** (1992) 31.
29. C. G. PITT, M. M. GRATZL, G. L. KIMMEL, J. SURLES and A. SCHINDLER, *Biomaterials* **2** (1981) 215.

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