The influence of bone formation on anchoring percutaneous devices with titanium fibre mesh flanges

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For man-made percutaneous devices (PD), it is known that anchoring will improve the clinical success. Previously, our Department has designed PDs that use a sheet of titanium (Ti) fibre mesh for anchoring. In nature, a very successful natural PD occurs, namely the tooth. Teeth are anchored in the alveolar bone. In the current study, we evaluated whether a sheet of (ectopic) bone can be made, and be used to anchor a skin-penetrating device. Using available tissue engineering techniques, sheets of Ti fibre mesh were loaded with osteoblast-like cells. These sheets, and non-loaded controls, were placed subcutaneously in 20 syngeneic rats. After four weeks 10 rats were sacrificed, and tissues were prepared for histology. On the other 10 rats, a percutaneous Ti bar was screwed. These rats were evaluated clinically up to eight weeks. Finally, also their tissues were prepared for histology. The results showed, that bone formation was only established in one cell-loaded implant, of the four-week group. Clinical evaluation, and the histomorphometrical data, showed no differences between cell-loaded and unloaded samples. We concluded that the combination of Ti mesh with rat bone marrow cells was not able to generate bone formation after subcutaneous implantation predictably. Thus, our original aim could not be met. On the other hand, our results did confirm the biocompatible behaviour of a PD equipped with a Ti fibre mesh anchoring flange.
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Introduction

A percutaneous implant or percutaneous device (PD) is an object made from a synthetic material that permanently penetrates through a surgically created skin defect [1]. There are numerous applications of PDs in medicine. Examples are catheters and dental implants. Other applications are for diagnostic purposes, direct monitoring of organ functions, attachment of limb prostheses, attachment of external hearing-devices, facilitating life supporting functions, artificial heart aids, etc. PDs provide a connection between the milieu interior and exterior of the body and thus, permanent skin penetration implicates the presence of a constant entrance port for bacteria. The PD exit site is generally regarded to be the ''Achilles heel'' of this type of device. The formation of a stable skin-implant junction at the exit-site will improve the success of these devices. In this perspective, it is known that anchoring of the PD will improve its final clinical success. For instance, the Department of Biomaterials at the University Medical Center Nijmegen has designed PDs that use a sheet of titanium (Ti) fibre mesh for anchoring. This PD was used with great success in the field of dialysis for renal patients, with the developed design currently in its last phases of pre-clinical testing [2–5].

Although new PD design strongly improves the clinical results, it is highly unlikely that it will provide the optimal solution. The improved behaviour is primarily directed toward delay and predictability of PD failure, more than on a complete prevention of failure. This suggestion is supported by the knowledge concerning the maintenance and failure mechanism of natural percutaneous structures, for example, teeth. Teeth penetrate the gingiva. The gingiva comprises that portion of the oral mucosa that covers the alveolar bone and surrounds the neck of the teeth. It consists of connective tissue and stratified squamous epithelium. The connective tissue of the gingiva contains collagen fibre bundles, the so-called gingival fibres. These fibres attach perpendicularly to the tooth, hereby tightly fastening the gingiva to the tooth. The periodontal ligament and the alveolar bone provide firmness and strength to the

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gingiva to withstand mechanical stresses, and thus are responsible for the maintenance of the pergingival passage.

Until recently, mimicking the presence of bone (or a gingival attachment apparatus) to achieve a natural exit site for PDs seemed a utopian scenario. However, with the current developments in biomedicine, especially in the field of ''tissue engineering'', this goal now seems feasible. With tissue engineering techniques, it should be possible to create an ectopic bone structure that can preserve its integrity over long periods of time.

We hypothesise that the performance of man-made PDs will increase, should the design of the PD reflect the tissues around ''nature's PD'', that is, the tooth. Therefore, the aim of this study was to investigate the possibilities of constructing ectopic bone in the flange of a PD, and to evaluate its effect on the performance of PDs in an animal model.

Materials and methods

The experiment was divided into the following phases:

1. Harvesting of bone marrow cells, and subculturing of the osteoblast-like cells.
2. Loading of these cells on Ti fibre mesh sheets.
3. Implantation of devices, equipped with the cell-loaded Ti fibre mesh sheets.
4. Analysis of wound healing and histological/histomorphometrical analyses.

Harvesting of bone marrow cells and cell culture

In this study, a total of 24 Fisher 344 male rats were used. Fisher rats are the preferred animals for this study because they form a syngeneic group (i.e. cells from one rat can be implanted in another rat, without evoking a specific immune response).

Bone marrow cells were isolated and cultured using the method described by Maniatopoulos [6]. Rat bone marrow cells were obtained from femora of four rats of 120–140 g. Femora were removed, and washed four times in α-minimal essential medium (MEM); (MEM Gibco BRL, Life Technologies B.V., Breda, The Netherlands) with 0.5 mg/ml gentamycin and 3 μg fungizone. Epiphyses were cut off and diaphyses flushed out with 15 ml α-MEM, supplemented with 10% fetal calf serum (FCS; Sigma, Chemical Co., St. Louis, MO, USA), 50 μg/ml gentamycin, 10 mM Na-β-glycerphosphate (Sigma), 50 μg/ml ascorbic acid (Sigma) and 10^{-8} M dexamethasone (Sigma). Cells were incubated in humidified atmosphere of 95% air, 5% Co_2 at 37°C. After seven days of primary culture, cells were detached using trypsin/ethylene diamine tetra-acetic acid (EDTA) [0.25% (w/v) trypsin/0.02% EDTA]. The cells were concentrated by centrifugation at 1500 rpm for 5 min and re-suspended in 15 ml complete medium with osteogenic supplements.

Loading of these cells on Ti fibre mesh sheets

A Ti fibre mesh was used, with a fibre-diameter of 40 μm, a volumetric porosity of 86% and a weight of 600 g m^{-2}. From this mesh, discs were cut, with a 20 mm diameter and a thickness of 1 mm. The meshes were sonicated for 10 min in isopropanol, and then sterilised by autoclavation for 15 min at 121 °C.

Subsequently, cells were seeded onto the Ti fibre mesh discs. After 4 h of attachment under gentle movement, cells were cultured on the Ti fibre mesh for one day. After culturing, meshes were washed twice in medium without additives, and used for implantation.

Implantation procedure with the cell-loaded Ti fibre mesh sheets.

Implants

The PD was made of KEL-F® (poly-chloride-trifluoro-ethylene). The sheet of Ti fibre mesh was held onto this device by a clamping ring. This ring was also made out of KEL-F® (Fig. 1). Onto the KEL-F® holding device, a Ti percutaneous cylinder could be screwed.

Twenty healthy young adult Fisher 344 rats (50–60 g) were used in the implantation study. National guidelines for the care and use of laboratory animals were observed. Surgery was performed under general anaesthesia. The animals were shaved, washed and disinfected with povidone–iodine. Parallel to the spinal column an incision of about 5 cm was made through the skin. Using blunt dissection a subcutaneous pocket was created. Thereafter, the subcutaneous part of the PD without the Ti screw was positioned in the subcutaneous pocket. Finally, the incision was closed using resorbable

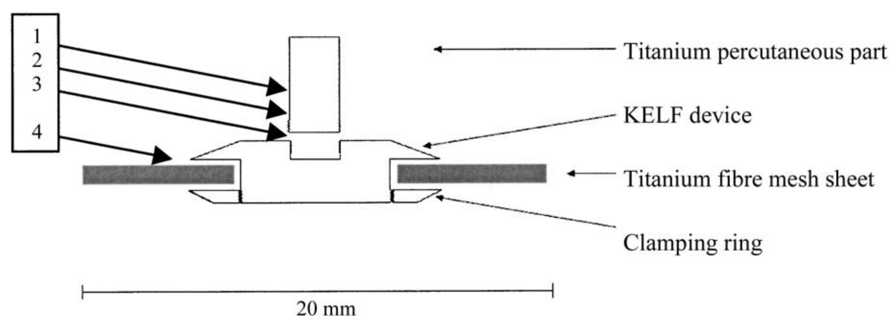


Figure 1 Schematic representation of the percutaneous device used in this study. The numbers indicate the scores for epidermal downgrowth: Score 1 = attachment of skin tissue to the PD (success); score 2 = downgrowth up to screw part; score 3 = downgrowth up to KELF ring; score 4 = downgrowth up to Ti fibre mesh (failure).

TABLE I Registration form of the clinical parameters as used to evaluate the exit-site of the PDs

		Score
Skin colour	Normal	1
	Pink	2
	Red	3
Drainage	No drainage/crust formation	1
	Hardly exudates around exit-site	2
	Hypertrophic granulation tissue	3
Granulation tissue	Absent	1
	Hardly present	2
	Hypertrophic granulation tissue	3
Skin retraction	Absent, skin closely around PD	1
	Present, holding ring visible	2
	Ti mesh visible	3

vicryl 3–0 sutures. The animals were divided into two groups:

Group I (10 rats). In group I, all rats received a PD without the percutaneous part. Five animals received a PD that was seeded with osteoblast-like cells, the other five received a PD without cells (controls). After a four-week period, the animals in group I were sacrificed and the implants, including all surrounding tissues, were retrieved for histological evaluation.

Group II (10 rats). In group II, also five rats received a PD that was seeded with osteoblast-like cells, and the other five received a PD without cells (controls). In these animals, four weeks after PD installation, a small incision through the skin was made, and the percutaneous part was attached to the holding device. These animals were kept for four more weeks. They were inspected every week. During these inspections the exit-sites of the PDs were investigated (Table I). The appearance of the exit-site was extensively registered using a systematic list, whereby a high total score represented a bad looking exit-site. At the end of the implantation period the animals were sacrificed and the implants, including all surrounding tissues, were retrieved for histological evaluation.

Analysis of wound healing and histological/histomorphometrical analyses

Histological procedures

At the end of the predetermined period of the experiments, the animals were killed by an overdose of Nembutal®. After retrieval, the tissue-covered implants were fixed in 4% buffered formalin for two weeks, dehydrated in a series of ethanol, and embedded in MMA. After polymerisation, sections for light microscopy were cut, using a modified diamond-blade sawing technique and then stained with basic fuchsin and methylene blue. For the histological and histomorphometric evaluation the following approaches were used (Table II) [7]:

1. The characteristics of the capsule surrounding the implant were rated qualitatively and quantitatively. The qualitative rating of the capsule consisted of a numerical rating of the tissue morphology (fibrous tissue and maturity) and cellularity (presence of fibroblasts, macrophages, and giant cells). The quantitative capsule classification consisted of a capsule thickness measurement based on the observed number of fibroblasts.
2. The characteristics of the interstitial tissue in the fibre mesh flanges were rated. This qualitative rating of the capsule will consist of a numerical rating of the tissue morphology (presence of bone-like tissue). For this purpose, light (LM) and scanning electron microscope (SEM) was done.
3. The distance of epidermal downgrowth along the percutaneous Ti bar was measured (group II only) (Fig. 1).

Scanning electron microscopy

After the preparation of histological sections, the remaining PMMA tissue blocks were polished until 1200 grit. These blocks were sputter coated with gold, and observed using a JEOL 6310 SEM, in back-scattering mode.

Statistical analysis

A χ^2- test used to compare clinical observation between animals in group two and also to compare the epithelial down growth along the implants.

The *t*-test used for the histomorphometric evaluation between groups I and II and also between animals in the same group. *P* values of less than 0.05 were considered to be statistically significant.

Results

Macroscopic and clinical findings

During the experiment one rat died from group II (control), due to too high a dose of anaesthetic. All of the other rats appeared to be in good health throughout the test period. At the second surgical session, the subcutaneous part of the implants could be easily detected by palpation. Further, it was observed that all subcutaneously placed implants showed good healing with the surrounding soft tissue.

The results of clinical evaluation are outlined in Fig. 2(a)–(d). Statistical analysis, using a χ^2- test, demonstrated that no significant difference existed for the clinical parameters except a border line significance for the drainage at the first week with $p = 0.047$.

Descriptive histological evaluation

Tissue blocks were cut into 10 μm histological slides. The four-week group and the eight-week group both showed excellent integration of the PDs into the surrounding tissues.

The tissue response to the Ti fibre mesh implants was relatively uniform for all animals. These meshes were surrounded by a connective tissue capsule containing 5–30 layers of fibroblasts and blood vessels. Frequently, inflammatory cells were observed within the immature fibrous connective tissue after four weeks (Fig. 3). However, after eight weeks the inflammatory reaction

TABLE II Histologic grading scale for soft-tissue implants

Reaction zone	Response	Score
Capsule quantitatively	Thickness rating	
	1–4 cell layers	4
	5–9 cell layers	3
	10–30 cell layers	2
	> 30 cell layers	1
	Not applicable	0
Capsule qualitatively	Reactive tissue is fibrous, mature, not dense, resembling connective tissue or fat tissue in the noninjured regions	4
	Reactive tissue is fibrous but immature, showing fibroblasts and little collagen	3
	Reactive tissue is granulous and dense, containing both fibroblasts and many inflammatory cells	2
	Reactive tissue 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
Interface qualitatively	Fibroblasts contact the implant surface without the presence of macrophages or foreign body giant cells	4
	Scattered foci of macrophages and foreign body cells are present	3
	One layer of macrophages and foreign body cells is present	2
	Multiple layers of macrophages and foreign body cells are present	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 noninjured regions	4
	Tissue in interstitium shows blood vessels and young fibroblasts invading the spaces, few macrophages may be present	3
	Tissue in interstitium shows giant 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 that may not only be related to the material to be tested	0

had decreased and capsule appeared to be thinner compared to four weeks (Fig. 4).

In group II, along the exit-site of the PDs, a downward migration of the epidermis along the Ti surface was always observed. The sinus tract was filled with keratin. No clear epithelial junction at the bottom of the sinus could be detected (Fig. 5).

Light microscopic evaluation of the cell-loaded implants, revealed that bone formation had only occurred in one sample, of the week-group (Fig. 6). The bone formation was characterised by the presence of multiple spheres in the pores of the Ti mesh. Bone always was in close contact to the Ti fibres, without intervening fibrous tissue layer. Morphologically, the appearance of these spheres was characterised by the presence of osteocyte-like cells embedded in a mineralised matrix.

Scanning electron microscopy

The bone formation in the histology often was hard to detect, due to the poor quality of the sections. Therefore, we confirmed all findings by performing SEM in a back-scattering mode on the polished tissue blocks. The SEM findings confirmed histology: only one sample in group I exhibited bone formation.

Histomorphometric evaluation

The histomorphometric results of capsule formation are given in Fig. 7(a) and (b). Statistical testing, using a *t*-test revealed that the fibrous capsule surrounding the subcutaneous Ti fibre mesh was quantitively as well as qualitatively not significantly different for both groups, with respect to the various implantation periods as well as both loaded *vs.* non-loaded.

The evaluation of the epidermal downgrowth (Fig. 7(c)) revealed that:

1. In the test group, the epidermis had migrated until the subcutaneous cuff.
2. In the control group, the epidermis had migrated until the subcutaneous cuff for one implant and for two implants the epidermis had migrated just to the beginning of the Ti bar.

Statistical analysis with a χ^2- test, revealed that there were no significant differences between the animals $(p = 0.11)$

Discussion

On the basis of previous investigations, we know that ectopic bone can be formed in sintered Ti fibre mesh and can be maintained subcutaneously. Therefore, the purpose of this study was to use this principle to create ectopic bone, and to determine whether the phenomenon of ectopic bone formation can be used to prevent epidermal downgrowth around a skin-penetrating device. This should lead to a more natural (tooth-like)

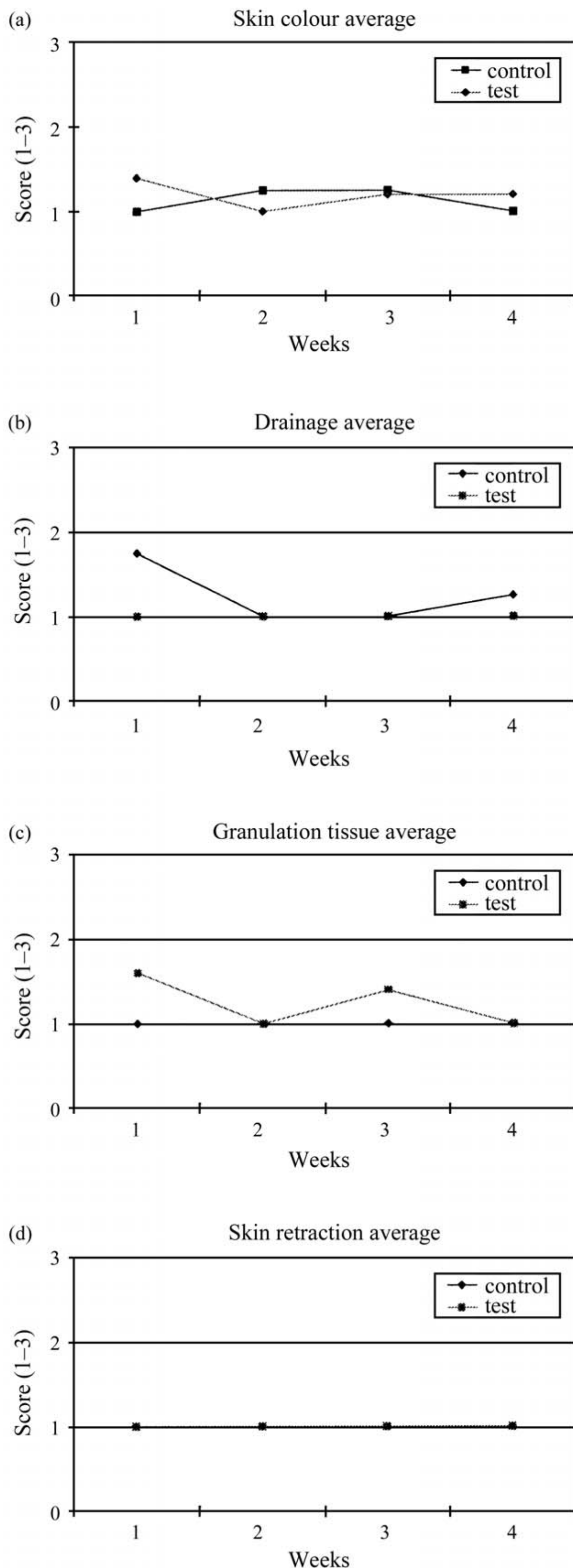


Figure 2 Graphs showing (a) the average for skin colour; (b) the average for drainage; (c) the average for granulation tissue; (d) the average for skin retraction.

anchoring for PDs, with an expected better clinical performance.

The results of this study confirmed the biocompatibility of Ti mesh. Tissue behaviour is excellent as demonstrated by the thin capsule thickness and absence of inflammatory cells in both cell-loaded and non-loaded meshes after eight weeks. The observed tissue reaction to the Ti fibre mesh was similar to other earlier experiments [2, 8–10].

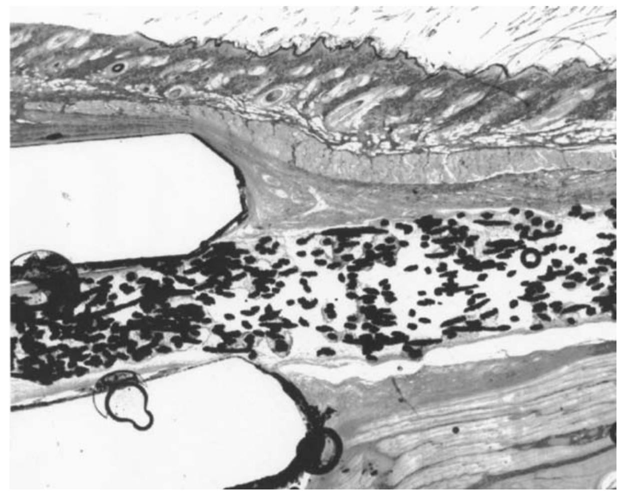


Figure 3 Light micrograph of the device after four weeks of healing. Control sample (no cells). Note the formation of a fibrous capsule around the KELF holding element, and the Ti fibre mesh (magnification 2.5 ×).

With regard to the bone formation, our experimental set-up was such as to mimic, as closely as possible, previous authors. For instance, we chose an incubation period of one day, as indicated by van den Dolder *et al.* (2002) [11]. They showed (*in vitro* and *in vivo*) that this period resulted in the highest calcium deposition after implantation. However, our histology and histomorphometrical measurements to quantify the bone formation, showed marked differences when compared with the previous studies. The number of ''positive'' implants (only 1 out of 10 cell-loaded implants in total) was significantly lower than other studies using similar methodology (Table III).

When comparing our study to other studies, as summarised in Table III, it seems logical to assume that the difference in bone formation could be due to the large difference in implant size. The Ti mesh in the current study was of the same thickness, but the increase of diameter for 6–20 mm would account for a 11-times larger total mesh volume. This also means an 11-fold increase of the total the surface area of the fibres.

Figure 4 Light micrograph of the device after eight weeks of healing. Control sample (no cells). Note the thinning of the fibrous capsule (magnification 2.5 ×).

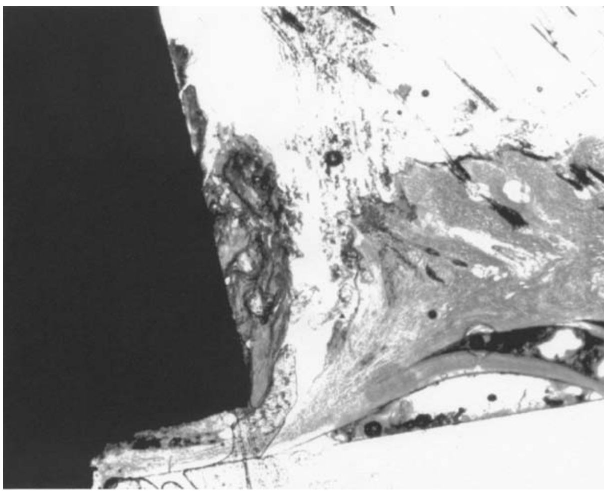


Figure 5 Light micrograph of a PD after eight weeks. Note the epidermal downgrowth along the percutaneous Ti bar (magnification 2.5 ×).

Therefore, it could well be that the loaded number of cells is too low. A high cellularity is known to enhance cell-to-cell contact and communication between cells. This is essential for phenotypic expression of cells *in vitro* [15]. A uniform and well-established distribution of cells inside the porosity of scaffold materials can be very relevant for the final *in vivo* bone inducing capacity of the construction [11].

The problem of implant size and cell number should be studied more extensively. Further, to enhance bone formation for future studies, several approaches could be followed. For instance, previous experiments with flow chambers proved to be successful [16]. Moreover, the scaffold material could be loaded with specific bone-inductive growth factors prior to implantation. Ti mesh loaded with bone morphogenic proteins (BMP) has been shown to adequately induce bone formation *in vivo* [17]. Also, it is known that bone formation can be greatly enhanced when the Ti mesh would have been equipped with a calcium phosphate (Ca–P) coating. In Table IV, comparison is given between plain Ti materials, and implants where growth factors added and Ca–P coating applied.

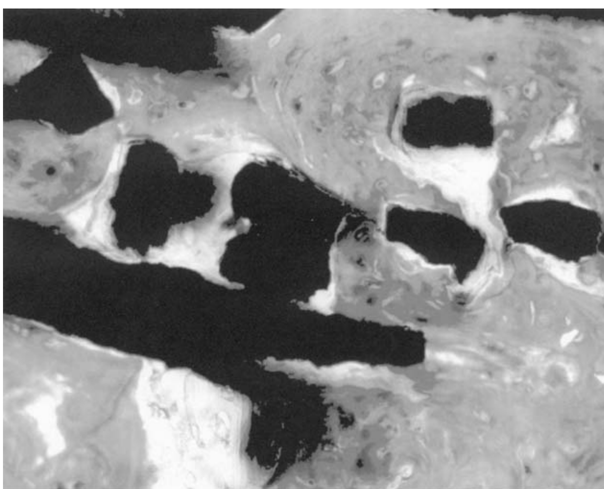
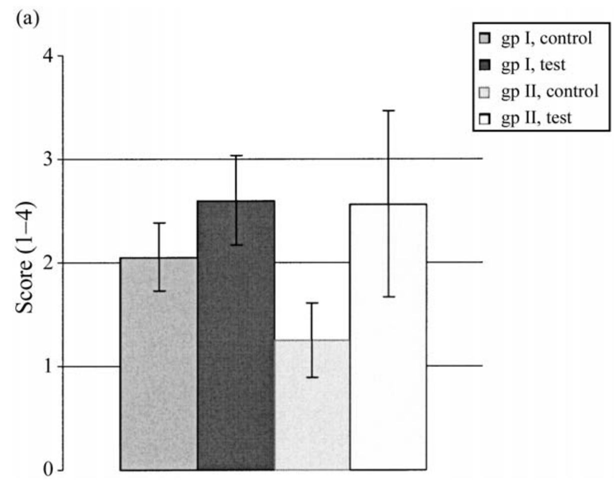
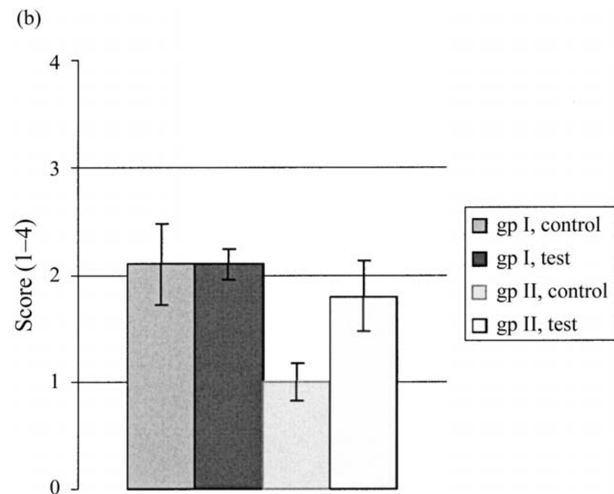


Figure 6 Light micrograph Ti fibre mesh loaded with cells, after four weeks. Note the bone formation in this sample (magnification 20 ×).





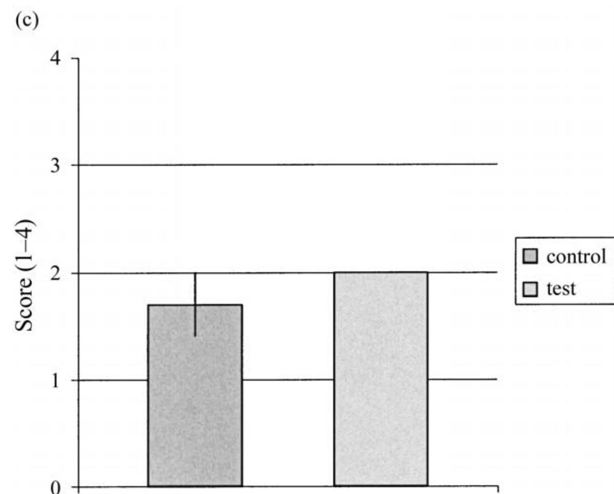


Figure 7 Graph showing capsule (a) quality and (b) quantity of the used PDs. (c) Graph showing the evaluation of epidermal downgrowth along the used PDs.

Of course, a direct comparison of the current study with various studies remains difficult, and is frequently impossible. For example, most studies differ in cell source as well as cell culture conditions. Some researchers use calvarial cells to examine the osteogenic capacity of the scaffold material [19–21], while others use bone marrow cells [19, 22–24]. In addition, different types of rats are used for cell retrieval, that is, Wistar [23, 24] and Sprague-Dawley [19, 25]. Also, there is great variation in the age of the rats in the that were used

TABLE III Comparison of the results of previous *in vivo* studies, to the current study

Study	Van den Dolder/[11]	Vehof/[12]	Van den Dolder/[13]	Vehof/[14]	Present study
Implamt diameter	6 mm	6 mm	7 mm	6 mm	20 mm
Time after seeding cells	One, four, eight days	Eight days	One day	Eight days	One day
Bone formation	More effective/ one day (subcutaneous)	Irregular calcified deposits (subcutaneous)	Bone formation (orthotopic site)	Bone in one sample at four weeks (subcutaneous)	Bone formation in one sample at four weeks (subcutaneous)

TABLE IV Comparison of the results of previous studies, on growth factors and Ca–P coated implants

Study	With TGF or BMP	With Ca–P coating
Van den Dolder/[18] Fibronectin/collagen (*in vitro*)	No difference	—
Vehof/[17] rhBMP-2 (*in vitro*)	Stimulate extracellular matrix calcification	—
Vehof [17] Ca–P coating (*in vivo*)	—	At eight weeks, significantly more bone formation
Vehof [17] Ca–P coating (*in vivo*)	—	At two + four weeks, significant bone formation
Vehof [17] BMP/Ca–P (*in vivo*)	10–40 days, more bone formation. However, same for coated and non-coated.	
Vehof [17] Ti–Ca–P/rhBMP-2 (*in vivo*)	Nine days, mineralized cartilage and bone formation	
Vehof [17] RhTGF/Ca–P (orthotopic site)	More dense bone than in Ti–CaP and Ti-implants. Ca–P, no enhancement of bone.	

by the various studies. All these discrepancies in primary conditions can have severe implications on the final results. The same is true for the cell culture conditions. Furthermore, in most experiments, dexamethasone is added as a supplement in order to enhance or induce the osteogenic differentiation of the cells. However, this supplement is added at different stages during cell culturing. When dexamethasone is provided for the first time after the first passage of rat bone marrow cells, the osteogenic expression of the cells is reduced [26]. Additional research will have to be conducted to answer this question. Moreover, the bone marrow cell population is very heterogenous in nature, which implies that different cultures can show different sensitivity. This will result in a differential response that cannot even be prevented by cell pooling or multiple experiments [27, 28]. We have to emphasise that this problem always will occur when primary cells are used and the experiments are repeated. This can only be prevented by using homogeneous cell populations, that is, cell lines. On the other hand, this just emphasises the problem with the reliable and standardised production of tissue engineered bone constructs when bone marrow is used as cell source.

Implantable devices have become important in medicine as well as dentistry and have played a central role in the restoration of function. Functional and safe skin-penetrating implants are urgently required for several types of applications in medicine. However, to develop skin-penetrating models for experimental studies as well as for clinically successful applications is a very difficult task. In dentistry, the hallmarks of implant failure are disruption of the perimucosal seal and loss of peri-implant bone, analogous to the destruction of soft and hard tissue seen in periodontitis. Once we have achieved proper bone formation, our skin-penetrating model could be very useful as a tool to study these periodontitis-related processes.

The mechanisms leading to implant failure have not yet been defined. It is well known that bacterial accumulations are probably the most significant etiologic factor in dental implant failure [29, 30]. Although, it may take longer for bacteria to colonize Ti surfaces, once colonized, the sequence of bacterial succession proceeds as on natural teeth [31]. In the dental implantology field, it is known that with failure due to infection, the microflora is similar to that which produces periodontal disease [32]. Thus, it is reasonable to expect that the pathogenic mechanisms also would be similar. Current thinking is that the destruction of periodontal tissue is a consequence of interactions involving endotoxin, cytokines, and cells of the periodontal regions [33–36].

We therefore feel that the only way to prevent the failure of PDs is to achieve total tissue integration. As suggested, tissue-engineering techniques could play a vital role in creating an environment for PDs that resembles the situation around teeth. In future experiments, tissue-engineering could not only be used to produce bone tissue, but also emphasis could lie on the development of gingival and periodontal ligament tissues. It may be assumed that by mimicking the anatomical situation of a junctional epithelium and a periodontal ligament this would be able to prevent bacterial infection before it occurs and thus eventually be more successful than simply control infections. Antibiotics will always have the disadvantage of recurring resistance and other bactericidal agents, like silver, are known to be cytotoxic and prevent proper tissue integration rather than enhance tissue healing. Finally, other mechanisms have to be studied accom-

panying tissue engineering. For instance, many studies have already shown that surface texturing can enhance the performance of PDs [37–39]. It is therefore apparent that considerable work in the future will have to be carried out in order to achieve an optimal situation in which a PD can be maintained in a stable situation for considerable time periods.

Conclusion

Our result did confirm the biocompatible behaviour of a PD equipped with a Ti fibre mesh anchoring flange. In addition, it was shown that the combination of Ti mesh with rat bone marrow cells was not able to generate bone formation after subcutaneous implantation predictably. Thus, our original aim could not be met since ectopic bone formation is difficult to establish in large sized Ti fibre mesh implants.

Acknowledgments

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References

1. A. F. VON RECUM and J. B. PARK, *Crit. Rev. Bioeng.* **5** (1981) 37.
2. Y. C. G. J. PAQUAY, J. E. DE RUIJTER and J. P. C. M. VAN DER WAERDEN, *J. Biomed. Mater. Res.* **28** (1994) 1321.
3. Y. C. G. J. PAQUAY, J. A. JANSEN and R. J. A. GORIS, *J. Invest. Surg.* **9** (1996) 81.
4. J. W. MONCRIEF and R. P. POPOVICH, in ''Peritoneal Dialysis'' (Kluwer Academic Publishers, Dordrecht, The Netherlands, 1988) p. 152.
5. Y. C. G. J. PAQUAY, J. E. DE RUIJTER, J. P. C. M. VAN DER WAERDEN and J. A. JANSEN, *Biomaterials* **17** (1996) 1251.
6. C. MANIATOPOULOS, J. SODEK and A. H. MECHER, *Cell Tissue. Res.* **254** (1998) 317.
7. J. A. JANSEN, W. J. A. DHERT, J. P. C. M. VAN DER WAERDEN and A. F. VON RECUM, *J. Invest. Surg.* **7** (1993) 123.
8. Y. C. G. J. PAQUAY, J. E. DE RUIJTER, VAN DER WAERDEN and J. A. JANSEN, *Biomaterials* **18** (1997) 161.
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. J. VAN DEN DOLDER, J. W. M. VEHOF, P. H. M. SPAUWEN and J. A. JANSEN, *ibid.* **62** (2002) 350.
12. J. W. M. VEHOF, J. VAN DEN DOLDER, J. E. DE RUIJTER, P. H. M. SPAUWEN and J. A. JANSEN, *ibid.* **64**A (2003) 417.
13. J. VAN DEN DOLDER, E. FARBER, P. H. M. SPAUWEN and J. A. JANSEN, *Biomaterials* **24** (2003) 1745.
14. J. W. M. VEHOF, P. H. M. SPAUWEN and J. A. JANSEN, *ibid.* **21** (2000) 2003.
15. S. L. CHENG, F. LECANDA, M. K. DAVIDSON, P. M. WARLOW, S. F. ZHANG, L. ZHANG, S. SUZUKI, T. ST JOHN and R. CIVITELLI, *J. Bone. Miner. Res.* **13** (1998) 633.
16. J. VAN DEN DOLDER, G. N. BANCROFT, V. I. SIKAVITSAS, P. H. SPAUWEN, J. A. JANSEN and A. G. MIKOS, *J. Biomed. Mater. Res.* **64A** (2003) 235.
17. J. W. M. VEHOF, Thesis (2001).
18. J. VAN DEN DOLDER, G. N. BANCROFT, V. SIKAVITSAS, P. H. M. SPAUWEN, A. G. MIKOS and J. A. JANSEN, *Tissue. Eng.* (2002) accepted.
19. S. L. ISHAUG RILEY, G. M. CRANE, A. GURLEK, M. J. MILLER, A. W. YASKO, M. J. YAZEMSKI and A. G. MIKOS, *J. Biomed. Mater. Res.* **36** (1997) 17.
20. S. L. ISHAUG RILEY, G. M. CRANE KRUGER, M. J. YASZEMSKI and A. G. MIKOS, *Biomaterials* 19 (1998) 1405.
21. M. P. LYNCH, J. L. STEIN, G. S. STEIN and J. B. LIAN, *Exp. Cell. Res.* **216** (1995) 35.
22. C. E. HOLY, M. S. SHOICHET and J. E. DAVIES, *J. Biomed. Mater. Res.* **51** (2000) 376.
23. L. X. BI, D. J. SIMMONS and E. MAINOUS, *Calcif. Tissue. Int.* **64** (1999) 63.
24. J. E. AUBIN, *J. Cell. Biochem.* **72** (1999) 396.
25. D. A. PULEO, *J. Cell. Physiol.* **173** (1997) 93.
26. P. J. TER BRUGGE and J. A. JANSEN, *Tissue. Eng.* **8** (2002) 321.
27. S. C. DIEUDONNE, J. VAN DEN DOLDER, J. E. DE RUIJTER, H. PALDAN, T. PELTOLA, M. A. VAN, T HOF, R. P. HAPPONEN and J. A. JANSEN, *Biomaterials* **23** (2002) 3041.
28. K. J. BURG, W. D. HOLDER, C. R. CULBERSON, R. J. BEILER, K. G. GREENE, A. B. LOEBSACK, W. D. ROLAND, P. EISELT P, D. J. MOONEY and C. R. HALBERSTADT, *J. Biomed. Mater. Res.* **52** (2000) 576.
29. M. ESPOSITO, J. M. HIRSCH, U. LEKHOLM and P. THOMSEN, *Etiopathogene. Eur. J. Oral. Sci.* **106** (1988) 721.
30. M. QUIRYNEN, M. DE SOETE and D. VAN STEENBERGHE, *Clin. Oral. Implants. Res.* **13** (2002) 1.
31. I. ERICSSSON, T. BERGLUNDH, C. MARINELLO, B. LILJENBERG and J. LINDHE, *ibid.* **3** (1992) 99.
32. M. G. NEWMAN and T. F. FLEMMING, *J. Dent. Edu.* **52** (1988) 737.
33. H. R. CREAMER, *Med. Hypoth.* **35** (1991) 115.
34. J. KATZ, J. GOULTSCHIN, R. BENOLIEL and Z. BEN-SASSON, *ibid.* **29** (1989) 251.
35. G. R. MUNDY, *J. Periodont. Res.* **26** (1991) 213.
36. R. PAGE, *ibid.* **26** (1991) 230.
37. B. CHEHROUDI, T. R. L. GOULD and D. M. AND BRUNETTE, *J. Biomed. Mat. Res.* **24** (1990) 1203.
38. B. CHEHROUDI, T. R. L. GOULD and D. M. BRUNETTE, *ibid.* **26** (1992) 493.
39. B. CHEHROUDI, T. R. L. GOULD and D. M. BRUNETTE, *ibid.* **34** (1997) 279.

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