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Histologic evaluation of the soft tissue response to sintered austenitic stainless steel fibre structures

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Recently it has been supposed that the soft tissue response to fibre mesh materials can be influenced by changing the flexibility and/or porosity of these materials. Therefore, to test this hypothesis, small sintered stainless steel (316L) fibre web implants with varying flexibilities and porosities were inserted subcutaneously into the dorsum of rabbits. The implants were left *in situ* for 15 weeks. Histological and tissue compatibility evaluations were performed. It was found that the best tissue reaction developed around the most porous fibre web material. Nevertheless, because of the possible occurrence of corrosion phenomena, the real existence of a relationship between implant porosity and connective tissue behaviour is still not clear.

1. Introduction

Considerable research activities have already been undertaken to create artificially a permanent percutaneous passage. The results of these experiments have demonstrated that reduction of tissue bed/implant motion reduces or eliminates the mechanical stresses at the interface between the implant and skin and benefits the long-term clinical success of the implant [1–6]. Consequently, various implant designs have been tested to reduce the interfacial stresses. In most of the approaches a flange is used for the subcutaneous anchoring of the percutaneous device. For the construction of these flanges extensive use has been made of a porous polyethyleneterephtalate (Dacron® velour) [7–10]. The theory was that connective tissue would infiltrate into the implant's pores and that stress reduction would be achieved by the formation of a strong mechanical connection between the collagen surrounding the Dacron® velour and that within the velour. Unfortunately, formation of mature well-organized connective tissue inside the interstices of the velour appeared to be impossible.

Given these disappointing results, it appeared appropriate to explore other methods and materials in order to improve the connective tissue response. For example, Ducheyne [11–12] developed a flexible porous titanium fibre structure for application in orthopaedic surgery. He demonstrated that bone grew unobstructed through this material. He supposed that this occurred because the flexibility of this material matched that of trabecular bone. Based on its performance in bone tissue, we decided to use this material for the stabilization of percutaneous devices in soft tissue [13]. The outcomes of our experiments demonstrated that these titanium fibre materials prolonged the longevity of the percutaneous devices and elicited a better tissue response with less macrophages than porous polymeric materials. We supposed that the reason for this favourable tissue reaction was the combination of the superior tissue characteristics of titanium with the typical mechanical characteristics of metal fibre products, like flexibility and stiffness. However, almost at the same time Campbell and von Recum [14] demonstrated that the connective tissue response to subcutaneously placed implants is mainly determined by the implant surface microgeometry and that variations in the bulk chemistry of the implant material have little influence. If this statement is indeed true, then it is also possible that the connective tissue reaction to our sintered titanium mesh is determined by its elastic properties and not the surface chemistry of this material. To test this hypothesis we performed a study [15] in which we evaluated the connective tissue response to three different sintered fibre-web materials: (1) sintered titanium fibre mesh; (2) sintered stainless steel (316L) fibre mesh; and (3) sintered Fe–Cr–Al alloy fibre mesh. Histological examination demonstrated that, after 12 weeks of implant tissue healing, all tested fibre web materials revealed a good connective tissue behaviour. These results supported the hypothesis of Campbell and von Recum, that, under the condition that no cytotoxic materials are used, the surface chemistry of an implant material is not so important. Therefore, we concluded that perhaps even a better tissue response can be achieved by optimizing the flexibility and porosity of the fibre mesh implants.

In light of the above mentioned findings, the purpose of this study is to compare histologically the connective tissue reaction to sintered stainless steel (316L) fibre web implants with varying flexibilities and porosities. These properties are created by using different fibre diameters for the production of the meshes.

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2. Materials and methods

2.1. Austenitic stainless steel fibre structure

For this study porous metal fibre implants made of stainless steel fibres type AISI 316L were used. These porous fibre structures are stable for autoclaving, are flexible and deformable. According to the information provided by the manufacturer the porous metallic fibre structures are fabricated by interengaging and intertwining a multiplicity of stainless steel fibres. After compaction the fibre structures are sintered to bond the metal fibres at their points of contact. The metal fibre used for the production of the structures can have a diameter varying from 2–22 μm, the amount of fibres is such that the structures can have a volumetric porosity between 65 and 95% and the thickness after compaction can vary between 0.1 and 30 mm.

Ten different materials were selected for the experiments. All the mesh materials are listed in Table I. The materials are coded and specified using the description a/b/c/d, where a is the metal type, b is the fibre diameter (μm), c is the panel weight (g/m^2), and d is the volumetric porosity (%). As can be seen in Table I, the volumetric porosity of all materials was 86%, the weight was 300 or 600 g/m^2, and the fibre diameter ranged from 2 to 22 μm.

At random chosen samples of all materials were inspected by scanning electron microscopy (SEM) and reflected light microscopy, also the surface composition was analysed by energy-dispersive X-ray analysis (EDAX, Philips 525). The flexibility of the materials was determined as deflection per unit load in a three-point bending test as performed with a thermal mechanical analyser (Mettler TMA 40). Additionally, with the same equipment, the thickness of the various mesh materials was measured. At least three specimens of each material were used for all the measurements.

2.2. Animals and implantation procedure

Five adult New Zealand White rabbits were used in this experiment. Five samples of each fibre mesh sheet were inserted subcutaneously in the dorsum of rabbits for 15 weeks. A total of 50 implants were placed, 10 in every rabbit. The implants measured 1 × 2 cm.

After ultrasonic cleaning and sterilization in an autoclave, the implants were inserted under aseptic conditions. Before implantation the rabbits were sedated by intramuscular injection of Hypnorm® (Duphar, Amsterdam) and the implantation site was shaved, depilated and scrubbed with Betadine®. For implantation of the implants, five longitudinal incisions were made on the left and right of the vertebral column, through the full thickness of the dorsum skin. One implant was inserted in each pocket. Subsequently, the wounds were carefully closed. To assure a complete and reliable randomization of the implant and to exclude the influence of implantation site, the various implants were allocated using the method of latin squares [16–17].

TABLE I Characteristics of fibre-web materials

Material	Mean thickness (mm)	Mean flexibility (μN)	Mean porosity (μm)
316L/2/300/86	0.238	1814.6	8.97
316L/2/600/86	0.451	242.5	14.82
316L/4/300/86	0.230	2756.7	15.99
316L/4/600/86	0.538	353.3	17.94
316L/8/300/86	0.265	4439.5	33.15
316L/8/600/86	0.541	1239.2	34.32
316L/12/300/86	0.233	8757.2	34.32
316L/12/600/86	0.538	1300.4	43.29
316L/22/300/86	0.277	3725	48.75
316L/22/600/86	0.529	673.3	43.68

2.3. Histological evaluation methods

At the end of the implantation period the rabbits were killed by injecting Nembutal® peritoneally. The implants, with their surrounding tissue and the skin, were excised immediately, fixed in 10% buffered formalin and embedded in methylmethacrylate. After polymerization, thin (about 10 μm) sections were cut, containing the implants as well as the surrounding tissues. The sections were made using a modified diamond blade sawing technique [18]. The sections were stained with basic fuchsin and methylene blue and investigated by light microscopy.

For the assessment of the soft tissue response to the implants, histological and histomorphometrical evaluations were performed. The histological evaluation consisted of thorough description of the observed tissue reaction. For the histomorphometry we used a histological grading scale, as shown in Table II, in which the histological characteristics of the capsule surrounding the implants and the tissue inside the interstices of the implants were evaluated by assigning scoring points. This evaluation method has already been described extensively in earlier papers [19, 20]. In summary, the evaluation of the surrounding capsule was semiquantitative and semiqualitative, while the evaluation of the interstitial tissue was only semiqualitative. The semiquantitative classification consisted of a capsule measurement on the basis of the observed number of fibroblasts. The semiqualitative rating of the capsule and interstitium consisted of numerically 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). Also the overall compatibility was determined by adding the scores of the surrounding tissue, interface and interstitium evaluation.

The histomorphometrical analysis of all sections was performed blind, and by three different evaluators.

3. Results

3.1. Materials characterization

Fig. 1a and 1b are scanning electron micrographs of the two mesh materials, which show the surface aspect of the metal fibre structure. The SEM analysis revealed that the surface of the metal fibres having a

TABLE II Histological grading scale for 316L mesh implants

	Response	Score
Capsule, semi-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, semi-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 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
Interstice, semi-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 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

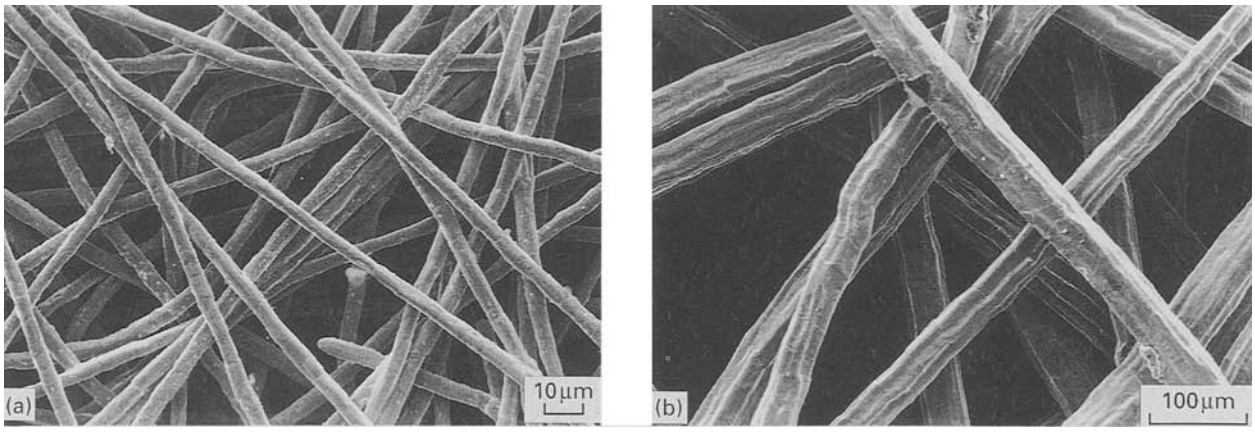


Figure 1 Scanning electron micrographs showing the surface aspect of 316L fibre structure: (a) 4 µm fibre; and (b) 22 µm fibre.

diameter of 2, 4 and 8 µm was rather smooth. On the 12 and 22 µm fibres shallow longitudinal grooves were observed.

Fig. 2 shows a cross-section of three fibre web materials and illustrates that the pore size of the material can best be described by the average of a pore range. The calculated average pore sizes for the various meshes are given in Table I.

The EDAX inspection showed that the most common elements detected on 316L mesh are chromium, nickel, molybdenum and iron.

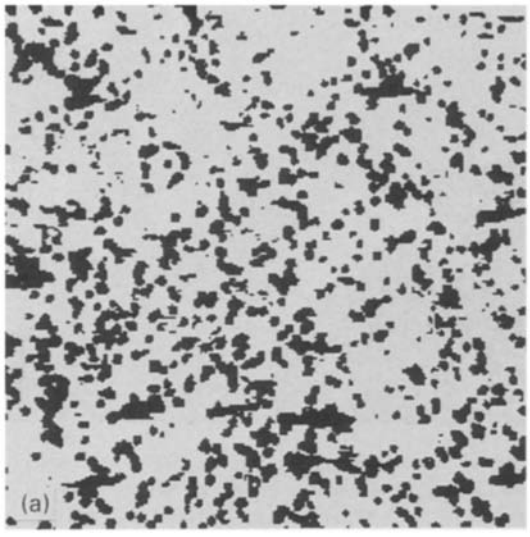
The measured mean flexibility and thickness for each of the mesh materials are listed in Table I.

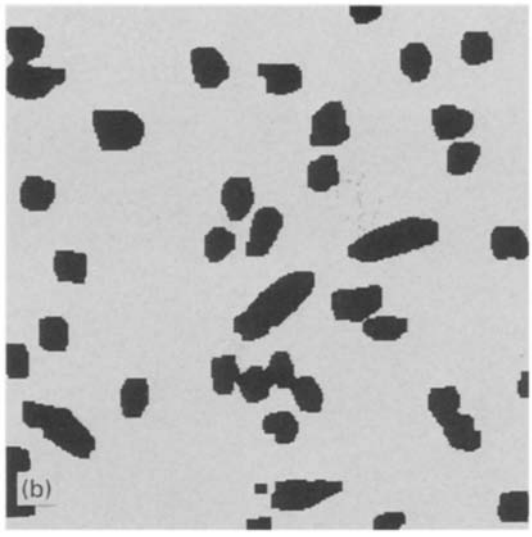
3.2. Macroscopic findings

Macroscopic inspection at harvesting of the specimens revealed that all implants were encircled by a thin adherent subcutaneous tissue capsule. Also, it appeared that four of the 2/300/86 and two of the 4/300/86 implants were fractured. In one rabbit, two implants (12/600/86 and 22/600/86) were merged.

3.3. Light microscopy

At 16 weeks, all wire mesh implants were surrounded by a thin to medium thin (5–45 µm) tissue capsule, containing fibroblasts and many inflammatory cells.





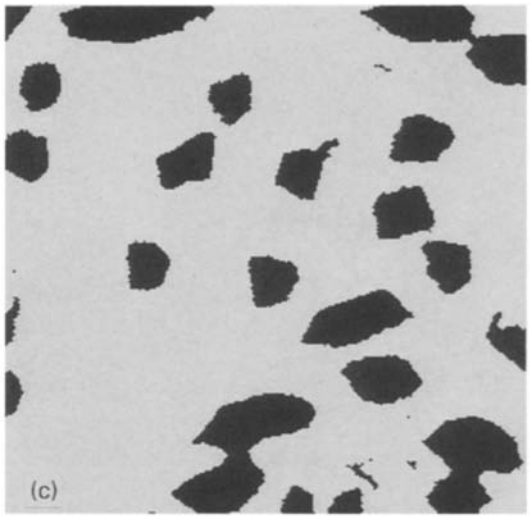


Figure 2 Cross-section of three fibre web materials showing the difference in pore size range: (a) 316L/2/300/86; (b) 316L/12/300/86; (c) 316L/22/300/86.

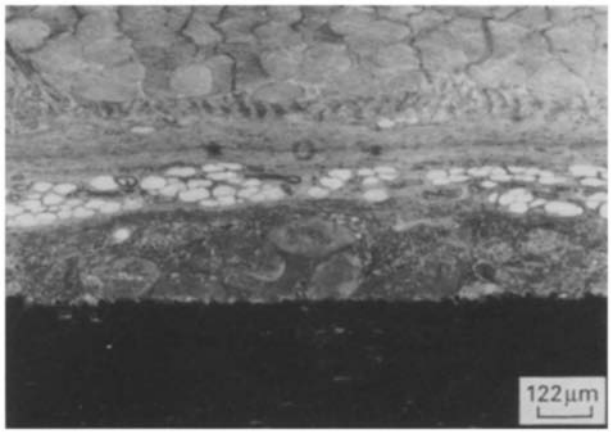


Figure 3 Light micrograph of 316L/4/600/86 web implant. The periphery of the implant is surrounded by several layers of macrophages and giant cells.

Although there was a striking similarity, the histological features of the tissue reaction to the various materials were not completely identical. About 50% of the mesh materials with a fibre diameter between 2 and 12 μm was lined around most of the periphery by one to several layers of macrophages and giant cells. Around these implants the tissue capsule only made direct contact occasionally at some areas with the implant surface (Fig. 3). On the other hand, in all sections of the 22 μm fibre diameter implants, no separation between the tissue capsule and the implant surface was observed (Fig. 4). Some of the implants showed evidence of capsule thickness reduction by the presence of fatty tissue. However, this fatty tissue could not prevent the formation of a separating inflammatory cell layer around 2–12 μm fibre diameter implants (Fig. 5). Further, in some specimens nerve bundles were observed immediately adjacent to the implant surface (Fig. 6).

Inside the majority of the mesh implants the porosity was filled exclusively with inflammatory cells (Fig. 7). Although, there was a suggestion that inside more open mesh implants (meshes with a fibre diameter between 8 and 22 μm) less inflammatory cells were present (Fig. 8).

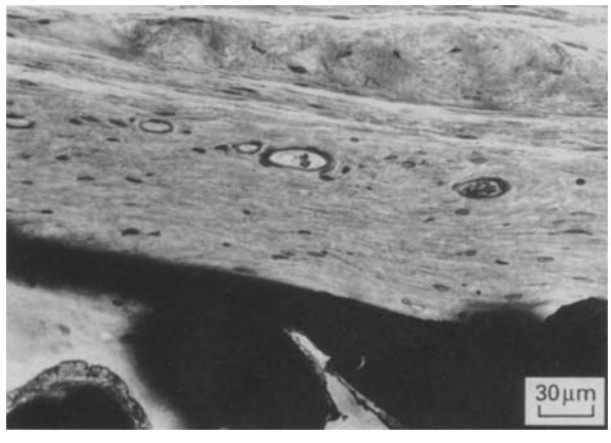


Figure 4 Light micrograph of 316L/22/300/86 web implant. No intervening layer of inflammatory cells is present between the implant surface and the fibrous tissue capsule.

In addition, in some of the most open meshes, occasionally areas were found where the interstitial tissue showed an invasion of young fibroblasts and blood vessels. Even fat tissue was seen extending through these implants.

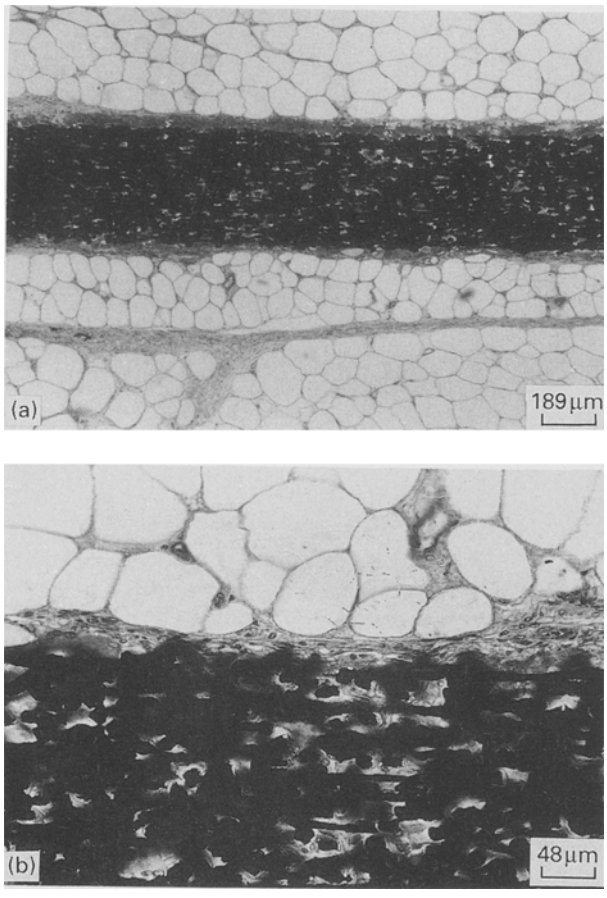


Figure 5 Light micrograph of 316L/4/300/86 web implant. The presence of fat tissue reduced the capsule thickness, but did not prevent the formation of several layers of inflammatory cells around the implant.

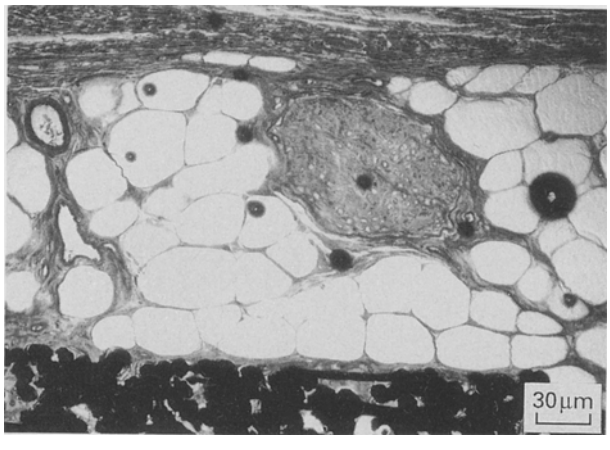


Figure 6 Light micrograph of 316L/8/600/86 web implant. Nerve bundle immediately adjacent to the implant surface.

3.4. Histomorphometrical comparison

Fig. 9 and Table III show all data of the histomorphometrical evaluation. Statistical testing of these data, using a one-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman–Keuls), revealed a significant difference ($p < 0.05$) in capsule quality between 4/300/86 mesh and 22/300/86 mesh. In addition, the analysis showed that a significant difference ($p < 0.05$) in capsule quality existed between 4/600/86 and 22/600/86 mesh. No significant

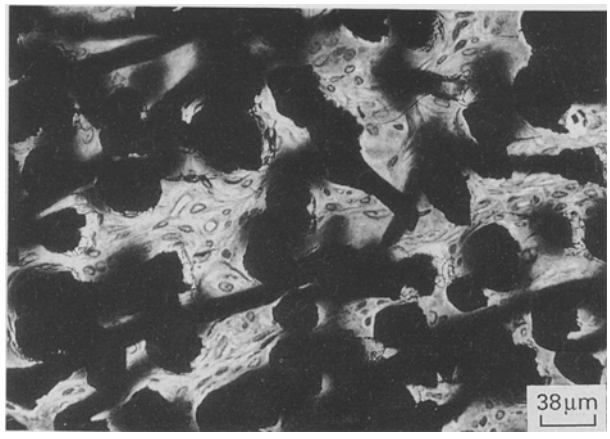


Figure 7 Light micrograph of 316L/8/300/86 web implant. There is macrophage accumulation inside the mesh implant. In addition, occasional small blood vessels are present between the fibres.

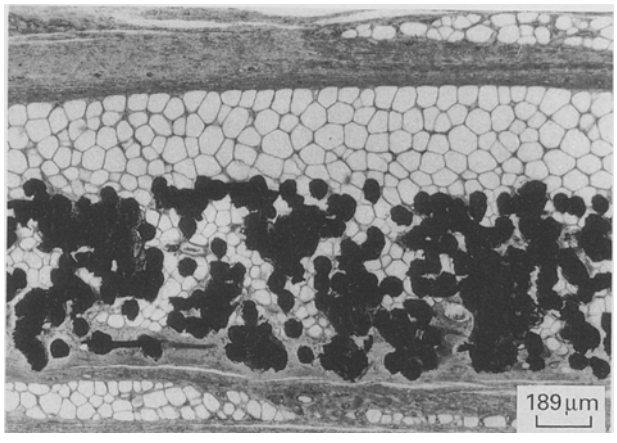


Figure 8 Light micrograph of 316L/22/300/86 web implant. Fat tissue is present inside the implants.

TABLE III Mean histomorphometrical data of the different implant types

Materials	Capsule quantity	Capsule quality	Interstitium quality
316L/2/300/86	2.60 ± 0.55 (n = 5)	1.40 ± 0.55 (n = 5)	1.00 ± 0.00 (n = 5)
316L/2/600/86	2.40 ± 0.55 (n = 5)	1.60 ± 0.55 (n = 5)	1.20 ± 0.45 (n = 5)
316L/4/300/86	2.40 ± 0.55 (n = 5)	1.00 ± 0.0 (n = 5)	1.00 ± 0.00 (n = 5)
316L/4/600/86	2.40 ± 0.55 (n = 5)	1.20 ± 0.45 (n = 5)	1.00 ± 0.00 (n = 5)
316L/8/300/86	2.60 ± 0.55 (n = 5)	1.40 ± 0.55 (n = 5)	1.40 ± 0.55 (n = 5)
316L/8/600/86	2.40 ± 0.55 (n = 5)	1.60 ± 0.89 (n = 5)	1.20 ± 0.45 (n = 5)
316L/12/300/86	2.50 ± 0.58 (n = 4)	2.00 ± 0.82 (n = 4)	1.50 ± 0.58 (n = 4)
316L/12/600/86	2.25 ± 0.50 (n = 4)	1.75 ± 0.50 (n = 4)	1.00 ± 0.00 (n = 4)
316L/22/300/86	2.75 ± 0.96 (n = 4)	3.00 ± 0.82 (n = 4)	1.75 ± 0.50 (n = 4)
316L/22/600/86	2.25 ± 0.50 (n = 4)	2.75 ± 0.50 (n = 4)	1.50 ± 0.58 (n = 4)

The number of implants that were studied of each material is shown between brackets.

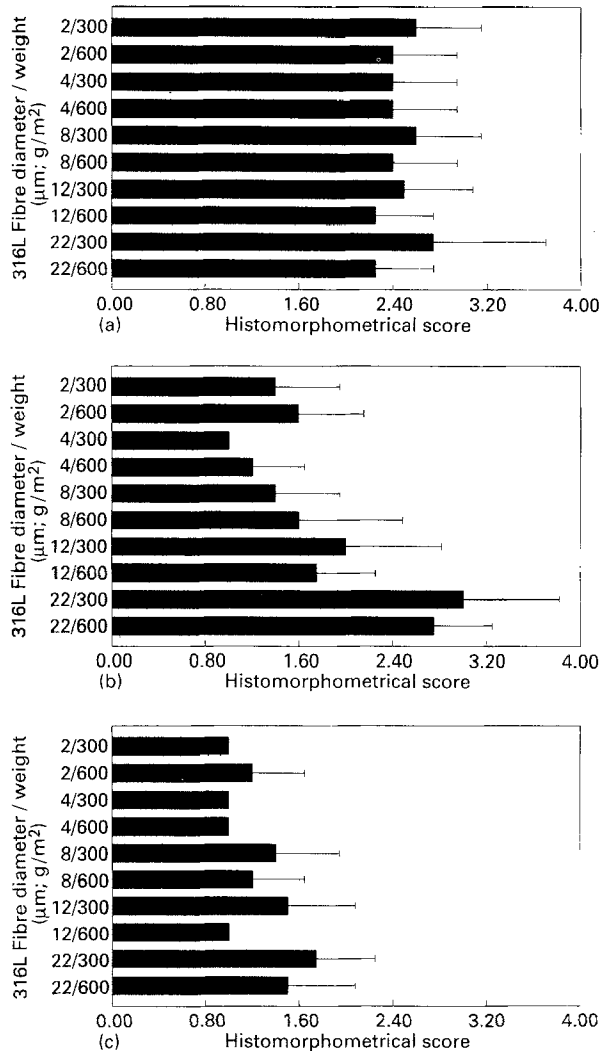


Figure 9 Comparative rating of: (a) capsule quantity; (b) capsule quality; and (c) interstitium.

difference was demonstrated in capsule quantity and interstitium quality between the various mesh materials.

To determine the possible existence of a relationship between tissue behaviour and porosity and flexibility of the mesh material, a simple linear regression test was applied. The statistical significance of the relationship between the two variables was examined by performing a t-test. The analysis revealed that a fair to moderate correlation existed between capsule and interstitium quality versus porosity. The computed correlation coefficient (r) for capsule quality and porosity was 0.5681 ($p = 0.00$), whereas the correlation coefficient for interstitium quality and porosity was 0.4046 ($p = 0.005$). Further, the analysis demonstrated the existence of a weak correlation between interstitium quality and flexibility ($r = 0.2959$, $p = 0.043$). However, no association could be identified between capsule quantity and porosity, flexibility and thickness of the various materials ($-0.2063 \leqslant r \leqslant 0.1336$).

4. Discussion and conclusions

The aim of this comparative study was to investigate further the relationship between implant porosity and connective tissue reaction.

Type 316L stainless steel appeared to be the most suitable material for these experiments. The choice was based on:

(a) commercial reasons: stainless steel is relatively inexpensive compared to titanium;
(b) the availability in very fine fibre diameters, which permits the production of meshes with varying flexibilities and porosities;
(c) the observed tissue compatibility [15, 21].

To our surprise, the results of the study described here provide only partial support for our earlier observations and hypothesis. It was demonstrated that a fair-to-moderate correlation exists between capsule and interstitium quality and porosity, with the best tissue development within and around the most open fibre web structure, while a very weak correlation exists between interstitium quality and flexibility, with the best interstitial tissue responses at the bottom of the flexibility gradient.

Two explanations can be given for the fact that these findings do not corroborate completely the previous studies. First, it can be supposed that the elastic and porous properties of the fibre web material which was used in these studies were already optimal. Therefore, changing the flexibility and porosity of the material does not improve, but in contrast deteriorates, the tissue reaction. Second, it is also possible that shear displacements at the interface implant/soft tissue disrupt the passive oxide film along the stainless steel surface. This will result in the release of metal ions [22]. That, indeed, such shear stresses are present around the fibre web materials can be concluded from the observed fracture of almost all thin and moderately flexible 2/300/86 meshes. Further, it cannot be ruled out that such a corrosion process can be increased by the pH decrease during the early stages of wound healing [23, 24]. When this corrosion phenomenon occurs, then the more unfavourable tissue response of the implants with a small fibre diameter is caused by a greater ion release as a result of the increased surface area of these meshes [25]. It is difficult to express which explanation is true. Certainly, McGeachie [26] found that small wire implants of titanium and stainless steel, inserted into mouse leg muscles, became encapsulated in a thin fibrous tissue capsule and that no observable differences existed between the two materials. On the basis of this observation, he concluded that there was no indication of any toxic effect for either material. Nevertheless, it has to be recommended that, to exclude interfering cytotoxic effects, for future studies only less-corrosion-suspectable materials are used.

In addition, our investigations indicated that implant flexibility and porosity had no influence on the capsule thickness. This finding is in agreement with our previous study [15] and with the observations of Coleman *et al.* [27], who reported that the thickness of the fibrous tissue capsule around implants is the result of the wound healing response to surgical trauma and has no relation with the chemical compatibility of the implant material.

Finally, there are other questions concerning the local tissue response, like the role of the difference in surface geometry between the various fibre diameters and the presence of fat cells, which remain unanswered at the moment.

In summary, the results of the present study indicate a correlation between porosity of the material used and tissue behaviour. Nevertheless, it is still not completely clear why the most porous of all tested wire mesh materials evoked less tissue reaction.

Acknowledgements

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