Tissue reactions to polyethylene implants with different surface topography

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This study investigates the importance of implant surface topography on soft tissue response. The tissue response in the rat abdominal wall to discs of low density polyethylene with smooth to coarse surfaces was evaluated after one, six or 12 weeks. Capsule thickness and immunohistochemical quantification of monocytes–macrophages were used as measures. The macrophage specific antibody ED1 was used for identification of newly recruited macrophages and the ED2 antibody for the mature tissue macrophages. The smoother surfaces gave a thicker capsule than the rougher surfaces, and at one week also larger total numbers of cells and ED1 positive macrophages at interface. The capsule thickness increased over time for the smooth and intermediate surface topographies. In contrast, the cell numbers generally decreased over time. In conclusion, a coarse surface elicited lesser tissue reaction compared with a smooth surface.

1. Introduction

The biocompatibility of a material depends upon several different factors related to the implant or to the tissue, such as material composition, implant design, surface properties, implant localization, state of the host bed, surgical technique and mechanical loading [1–4]. It is conceivable that the surface properties play a key role for biocompatibility [5–8]. Several *in vivo* studies have been done in respect to surface-related bone reactions and in these studies a relation between rough implants and better bone (in)growth was found [9–15]. However, there is still no consensus on the ultimate surface structure. The importance of various surface topographies for soft tissue reactions is more sparsely investigated, but it has been demonstrated that the topography affects the tissue reactions [16–19]. When modifying the surface topography it is conceivable that various factors are altered such as surface chemistry and surface energy [2], which in turn may affect both the molecular and cellular events at the surface. This is supported by a previous study showing that surface topography influences protein adsorption [20].

Various parameters have been used to evaluate the tissue response to different implanted materials. The macrophage is considered to be of major importance in the inflammatory response to an implanted material and is therefore a valid parameter for biocompatibility assessment [21, 22]. Dijkstra *et al.* [23] have identified subpopulations of macrophages in the rat by using monoclonal antibodies that allow unambiguous identification of macrophages and their subpopulations [24]. We and others [25–29] have in previous studies used the identification and quantification of these macrophage subpopulations as measures of implant induced soft tissue reactions.

This study addresses the soft tissue reactions induced by polyethylene implant surfaces that differ with respect to surface topography. Immunohistochemical quantification of the macrophage subpopulations was used as a measure. The implantation periods were chosen to be one, six or 12 weeks to ensure a reasonable stable tissue organization.

2. Experimental procedure

2.1. Animals

Male Sprague–Dawley rats ($n = 24$), weighing 200–250 g, fed on standard pellets and water *ad libitum*, were used. The rats were anaesthetized by intra peritoneal injections of 1.0 ml 100 g^{-1} body weight of a solution containing sodium pentobarbital (60 mg ml^{-1})

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and NaCl (9 mg ml^{-1}) in 1 : 9 volume proportions. The experimental protocol was reviewed and approved by the Animal Ethics Committee in Lund, Sweden.

2.2. Implants

A commercial low density polyethylene (LE 6607-PH) was dissolved to a 10% (w/w) solution in *o*-xylene (KEBO) by heating for 30 min at 170 °C. The polyethylene solution was cast in glass petri dishes that were either smooth or ground with 320 and 100 grains mm^{-2}, respectively. This resulted in a set of three surfaces with different structures: smooth, intermediate (irregularities of $< 10\,\mu m$; 320 grit) and coarse (irregularities of 10–50 μm; 100 grit). The solvent was evaporated at 110 °C for 24 h in a circulating air oven and at 40 °C for 24 h in vacuum. Before removing the films from the glass, they were hydrated for 24 h in distilled water and thereafter wiped dry. Circular disc specimens (diameter 5 mm, thickness 1 mm) were cut from the polymer films and used as implants [30]. The glass-facing sides of the films were examined as well as the air-facing side on the smooth film. Before insertion the implants were cleaned and sterilized in ethanol, rinsed and kept in sterile saline until surgery.

2.3. Scanning electron microscopy (SEM)

Film samples of non-implanted material were mounted on stubs and coated with 20 nm of Au–Pd. An ISI-100A microscope was used to perform the SEM analyses.

2.4. Implantation procedure

Details of the implantation procedure have been described previously [30]. In brief, the rectus abdominis muscle sheath was opened and the muscle moved laterally. Two implants were inserted on either side of the linea alba. The implants were placed outside the peritoneum without injuring the peritoneal membrane. For each implantation time point eight rats were used. In each rat, four implants were inserted (Fig. 1). The site for the implants was systematically alternated. Three of the implants had their modified surfaces placed towards the muscle tissue with the air surface facing the peritoneum. The fourth implant had the smooth surface placed towards the peritoneal membrane, making it possible to evaluate the tissue effects of smooth surface orientation as well as the tissue effects of air versus glass contacting surfaces at the casting procedure (Fig. 1). The rectus abdominis muscle was slipped back to cover the implant and a suture was placed in the muscle sheath to secure the position of the implant.

2.5. Tissue fixation

After one, six or 12 weeks the animals were re-anaesthetized and the implants with surrounding tissue were removed *en bloc*. The specimens were washed in ice-cold phosphate buffered saline (PBS, pH 7.4),

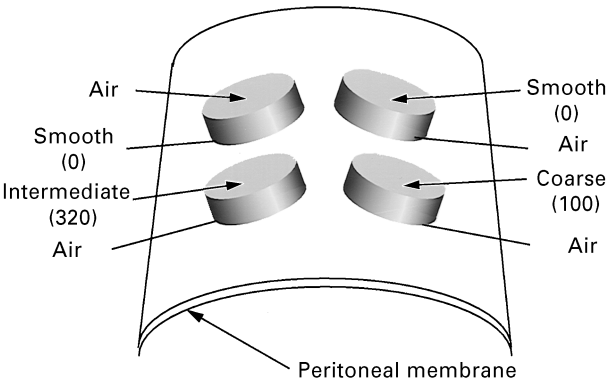

Figure 1 Schematic figure of the sample placement when implanted on the peritoneal membrane of the rat. The side of the implant facing the peritoneum is denoted "down" and the muscle facing side "up". The variations allowed several comparisons: (a) the tissue effects of different surface textures for smooth, intermediate and coarse surfaces facing the muscle; (b) the importance of the surface orientation for the tissue response, comparing a smooth surface facing either the muscle or the peritoneal membrane, and (c) the importance of the casting procedure for the tissue response, comparing a smooth surface versus an air surface when facing the muscle.

embedded in Tissue Tek® embedding medium 4583 (Histolab Products AB, Sweden) and snap frozen for 30 s in 2-methylbutane at − 70 °C. Implants with surrounding tissue were sectioned in a cryostat (6 μm in thickness) and collected on chromium-alum treated slides and allowed to air dry. The slides were kept at − 70 °C until stained.

2.6. Immunohistochemistry

The staining procedures for the macrophage subclasses ED1 and ED2 were done according to Rosengren *et al.* [29]. In brief the staining procedure was as follows. After removal of the endogenous peroxidase activity and blocking of unspecific bindings the slides were incubated with primary antibodies. The primary antibodies used were mouse anti-rat ED1 and ED2 (Serotec). The primary antibodies were exposed to a biotinylated horse anti-mouse IgG secondary antibody. The sections were then incubated with Vectastain ABC peroxidase standard PK-4000 (Vector). The presence of peroxidase was detected using 3-amino-9-ethyl-carbazole (Sigma). Thereafter the sections were counterstained in Mayers HTX. For the control sections, mouse monoclonal antibodies directed to human cell surface antigens were used as primary antibodies.

All images were obtained using a Kodak DSC-200 digital camera (Rochester, New York), mounted on a Nikon FXA microscope using bright field microscopy. The images were retrieved and marked using Photoshop 3.05 software (Adobe) on a Macintosh 8500 computer (Apple) and printed on a Codonics (NP-1600 M) sublimation printer.

2.7. Morphometry

Sections from eight implant interfaces representing each surface modification and each evaluation period

were evaluated by the same person (AR), who did not know which group an individual specimen belonged to. The number of cells was determined by manual counting of positive cells on sections stained for ED1 and ED2, respectively ($n = 8$ for each evaluated biological parameter at each time point). Further, the number of cell nuclei (counterstained cells) was used as a measure of the total number of cells, which thus includes macrophages, fibroblasts and other cells [29]. The quantification was done in a Leitz microscope in bright field mode at $25\times$ magnification. A 10×10 ocular square grid, where each square covered a $42 \times 42\,\mu m$ large area, was superimposed at the centre along the tissue border adjacent to the implant surface. The thickness (micrometers) of the reactive capsule was determined using the grid and was defined as the distance between the tissue border adjacent to the implant and the muscle border. The number of cells in the measured capsule were manually counted in five rows of squares from the implant surface to the border of the muscle. The cell numbers are given in numbers per millimetre squared based on the actually counted area. In order to quantify the tissue reactions immediate to the implants the number of cells in the interfacial area was defined as the row of five squares along the implant surface. Within these squares the number of cells were counted as described for the capsule.

2.8. Statistics

Non-parametric statistics was used in this study using the Statview 4.5 (Abacus Concepts) for the Macintosh. The effects of surface roughness and changes over time were evaluated using Kendall ranks correlation test. Corrections due to multiple comparisons were done with the sequentially rejective Bonferroni test [31]. The relevant p-values corresponding to a single comparison at $p < 0.05$ are given for each comparison. For evaluation of the relative effects of orientation (smooth surfaces facing muscle or peritoneum) and surface chemistry (air- versus glass-contacting surfaces) additional variance analysis (ANOVA) was done using the same software. All values are presented as boxplots showing median values with interquartil range.

3. Results

3.1. Surface structure

The surfaces with different roughness are shown in Fig. 2a–d. The smooth surface was very even and no defects could be seen (Fig. 2a), but the air-facing side showed a weak undulation (not shown). The surface with intermediate roughness showed a homogeneous topography with irregularities primarily less than 10 μm (Fig. 2b). On the coarse surface irregularities of varying sizes were seen but these were mainly between 10 and 50 μm (Fig. 2c). Small pits, $< 1\,\mu m$, occurred on the surface of the irregularities (Fig. 2d). These pits may be derived from the loosening of the films from the glass moulds.

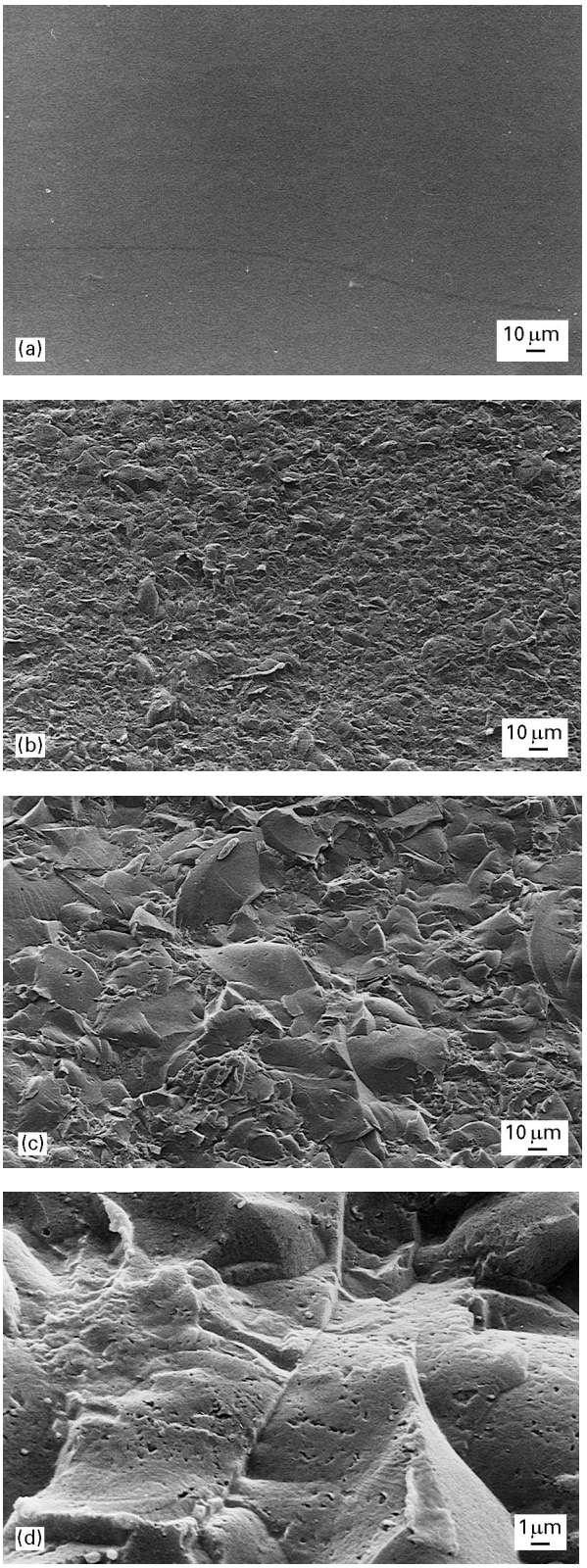

Figure 2 Scanning electron micrographs showing the various glass-facing surfaces before implantation: (a) smooth, (b) intermediate, (c) coarse, and (d) coarse.

3.2. General light microscopical observations

At all time points, irrespective of surface topography, the foreign body reaction consisted of an inner cell-rich zone where monocytes–macrophages with ED1 immunoreactivity predominated (Fig. 3a–d), but also ED2 positive cells and other cells (most likely

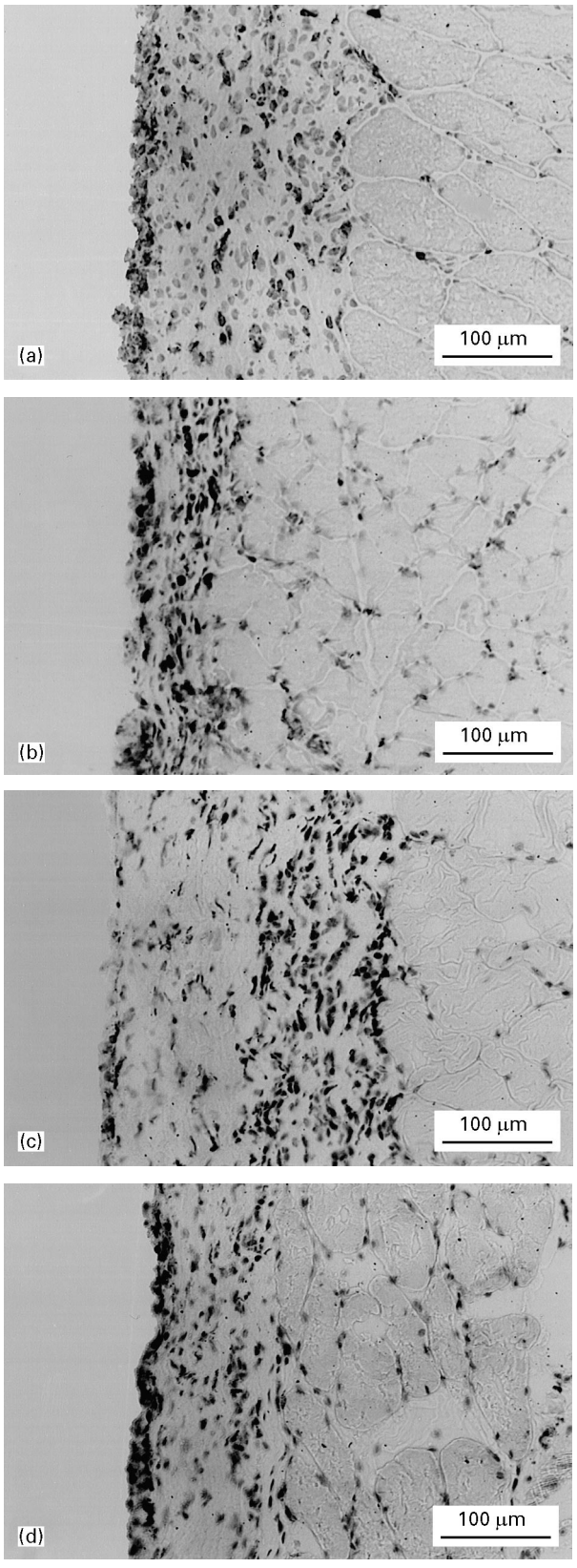

Figure 3 Microphotographs of sections taken from the tissue adjacent to implants inserted in rat abdominal wall after one or 12 weeks. The immunohistochemical staining specific for ED1 cells appears black and cell nuclei grey. The implant is to the left. (a) After one week implantation time; smooth surface, (b) after one week implantation time; coarse surface, (c) 12 weeks implantation time; smooth surface and (d) 12 weeks implantation time; coarse surface.

fibroblasts) could be observed. More distantly from the implant surface the ED2 positive cells were more abundant as compared with the ED1 cells. Further, other cells increased in number and blood vessels intervened in the tissue. In general, the total number of cells as well as the ED1 and ED2 positive cells decreased in number over time at all investigated surfaces both at the interface and throughout the reactive capsule.

3.3. Capsule thickness

At 12 weeks the foreign body response displayed a statistical significant correlation with decreasing capsule thickness by increasing surface roughness, $p = 0.0025$ (Fig. 4). A similar tendency was seen at one and six weeks, but without reaching statistical significance. The capsule increased in thickness over time for both the smooth ($p = 0.0001$) and the intermediate surfaces ($p = 0.021$).

3.4. Cellular density

In the interfacial zone the ED1 positive cells were more numerous as compared with the ED2 positive cells at all examined surfaces and times. At one week, but not at six or 12 weeks, the macrophages with ED1 immunoreactivity decreased in number with increasing surface roughness, $p = 0.0144$ (Fig. 5). The total cell number at interface showed a similar tendency as for the ED1 positive cells. Thus, there was an association between increasing surface roughness and decreasing cell numbers ($p = 0.0079$) at one week but not at six or 12 weeks (Fig. 6). When evaluating the kinetics of the tissue response at interface the ED1 and total number of cells decreased over time at the smooth ($p = 0.0014$; $p = 0.0005$) and the intermediate surfaces ($p = 0.0040$; $p = 0.0001$) but not at the coarse surface (Figs 5 and 6). The intermediate surfaces also showed a decrease in ED2 positive cells ($p = 0.0010$) over time (not shown).

In general, the cell distribution pattern in the reactive capsule was similar to that of the interfacial area, but without a statistical significant correlation between cell number and surface roughness. However, when evaluating the kinetics of the cellular composition in the capsule, the smooth ($p = 0.0021$) and the intermediate ($p = 0.0008$) surfaces exhibited a decreasing number of ED1 positive cells over time (Fig. 7). The development over time for the coarse surface was similar but without reaching statistical significance. The ED2 cells showed a similar pattern without reaching statistical significance. The total cell number in the capsule decreased significantly with time for the smooth ($p = 0.0001$) and the intermediate ($p = 0.0001$) surfaces (Fig. 7).

3.5. Importance of orientation of the implant and casting procedure

In the orientation experiments, where smooth surfaces had been in contact with either muscle or peritoneum the ANOVA test could not detect any differences in the tissue response at any time point (see smooth up and smooth down in Figs 4, 5 and 6). It further showed no significant difference in tissue response between smooth surfaces either made in contact with glass or

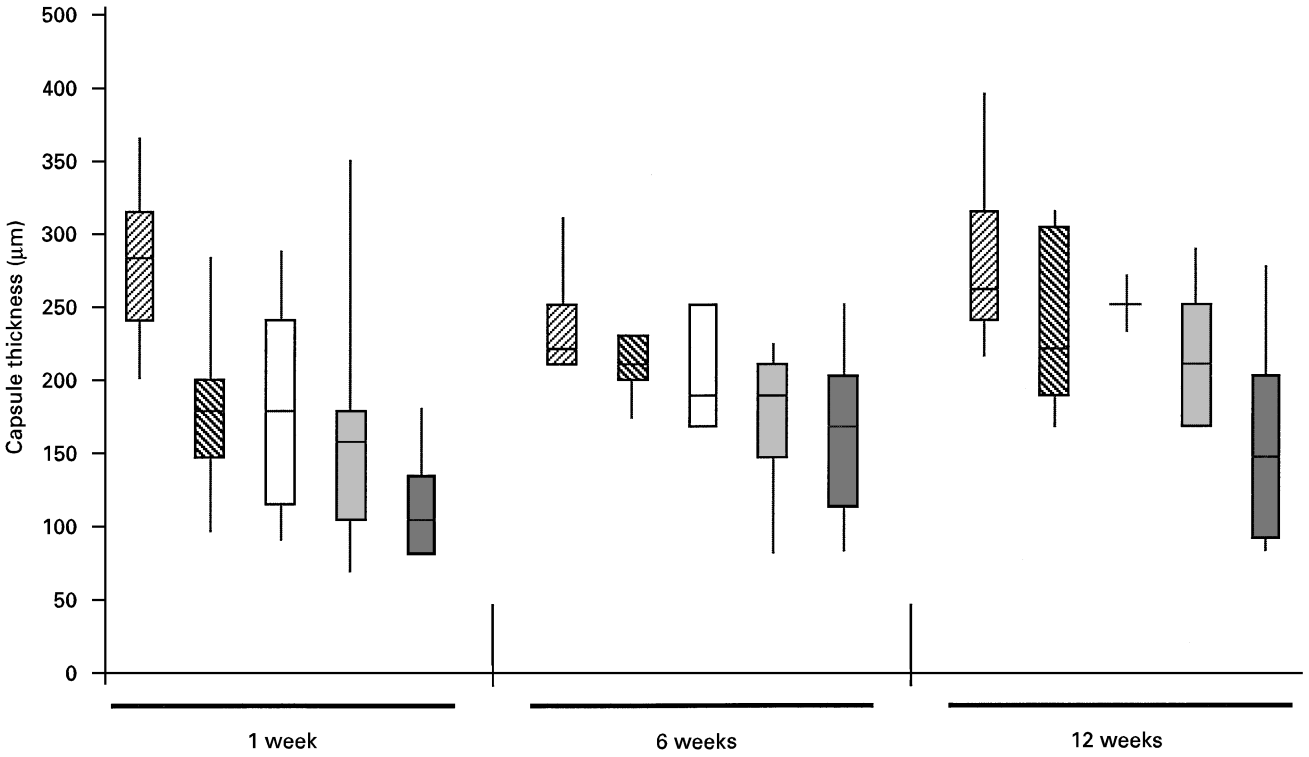

Figure 4 Box plot showing the capsule thickness after one, six or 12 weeks of implantation for the various surfaces and orientations studied (see Fig. 1). Smooth down ▨; air up ▧; smooth up □; intermediate up ▩; coarse up ■. The box represents the 25 and 75 percentiles and the median value is indicated by the horizontal line within the box. The ten and 90 percentiles are indicated by the whiskers.

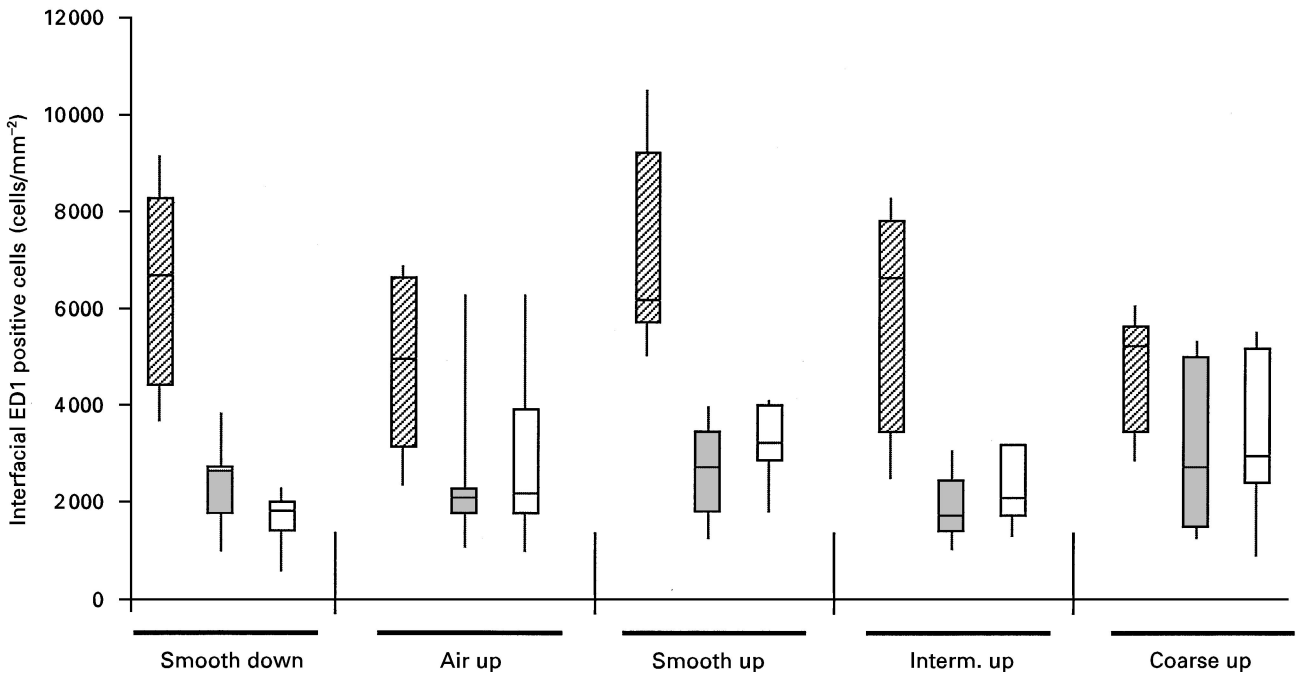

Figure 5 Box plot showing the ED1 cell density at the interface after one ▨, six ▩ or 12 □ weeks for the various surfaces and orientations studied (see Fig. 1). The box represents the 25 and 75 percentiles and the median value is indicated by the horizontal line within the box. The ten and 90 percentiles are indicated by the whiskers.

in contact with air (see air up and smooth up in Figs 4, 5 and 6).

4. Discussion

Implant factors hypothesized to govern the tissue response include: movements of implant, structure and shape of implant, surface chemistry and physical interactions [32, 33]. Studies on the soft tissue reactions to implant surface structures *in vivo* have given diverse results [17, 19, 25]. However, consensus from such studies seems to be that a textured or porous surface is preferable over a smooth one.

In the present study we have investigated the importance of various polyethylene implant surface textures on the soft tissue response. Thus, various surface

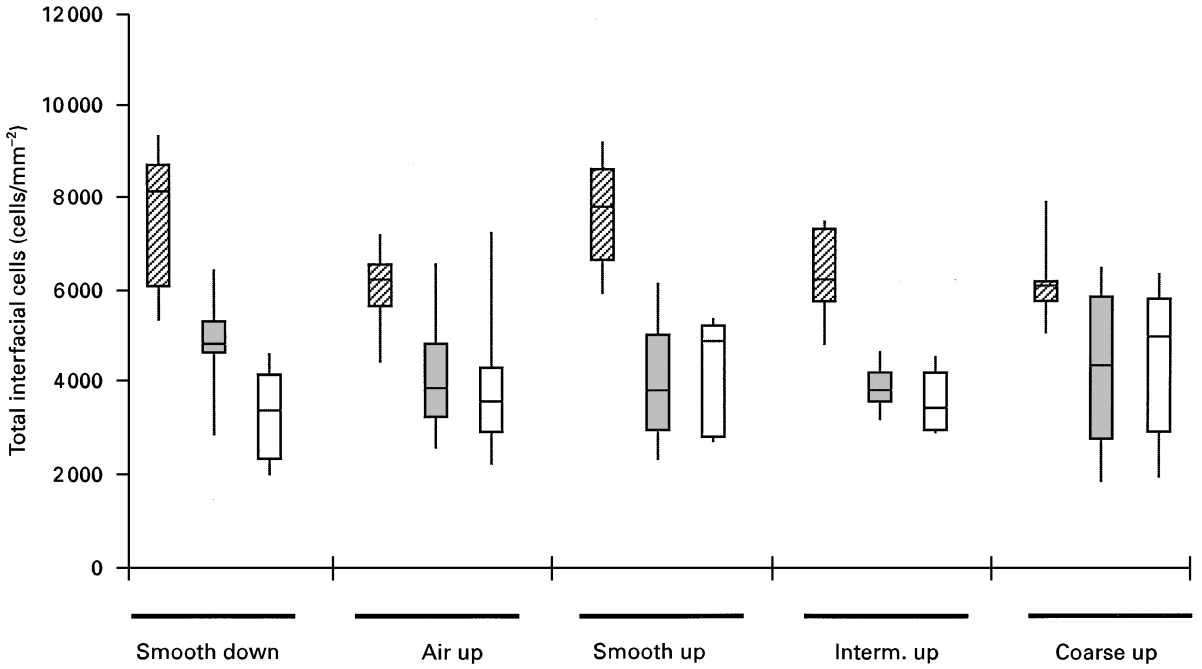

Figure 6 Box plot showing total cell density (including macrophages, fibroblasts and other cells) at the interface after one ▨, six ▩ or 12 □ weeks for the various surfaces and orientations studied (see Fig. 1). The box represents the 25 and 75 percentiles and the median value is indicated by the horizontal line within the box. The ten and 90 percentiles are indicated by the whiskers.

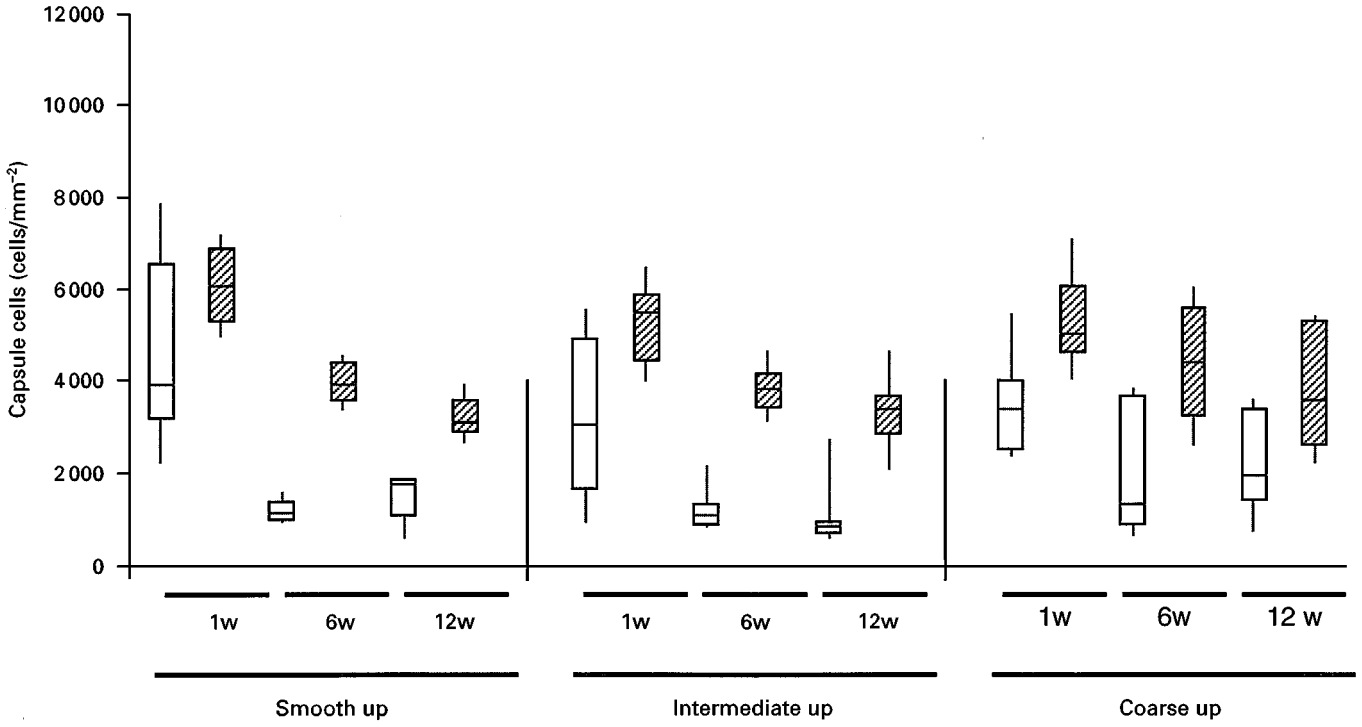

Figure 7 Box plot showing the density of ED1 positive cells ▨ and total cells □ (including macrophages, fibroplasts and other cells) in the reactive capsule after one, six or 12 weeks for smooth, intermediate and coarse implants (see Fig. 1). The box represents the 25 and 75 percentiles and the median value is indicated by the horizontal line within the box. The ten and 90 percentiles are indicated by the whiskers.

topographies were created on polyethylene-film by casting on glass surfaces that were either smooth, or made coarse by grinding. The analysis of the outcome is based on comparisons of some biological parameters for different surfaces. The sequential rejective Bonferroni test was done to eliminate false differences due to multiple comparisons [31]. The evaluations of the tissue response in this study are based on manual cell counting of frozen tissue sections where the implants are removed before sectioning. The manual cell counting procedure has limitations in the numbers of cells and samples that can be evaluated. The benefits of manual counting are, for example, the variations in stainability and difficulties in correctly counting cells in clusters, that are common with computerized image analysis, are avoided. Subjective biases can efficiently

be eliminated by doing the counting without knowledge of sample origin. A possible, but not probable, cause for the observed differences in tissue response to the various surface topographies is that the interfacial cell layers at especially the coarse surface may have been torn from the sample when removing the implants. However, neither the ED1 positive cells, that are invariably found at the materials–tissue interface, were fewer at the coarse surfaces nor were the ED2 positive cells, normally found at some distance from the material, increased.

Differences found in this study between the individual experimental groups were generally small. However, when using non-parametric correlation tests some clear trends were found. Thus, at all times the smoother surfaces displayed thicker capsules than the rougher, and at one week an increased total cell density and an increased ED1 positive macrophage density at the interface were observed. Further, the cell number generally fell over time. The capsule thickness, however, increased over time, at least for the smooth and intermediate surfaces. For the coarse surface it is unclear if there is a further increase after the initial six weeks. With regard to the capsule thickness this study corroborates earlier studies, showing that surface topography modulates the formation of foreign body reaction around soft tissue implants. In general, smoother surfaces induce more capsule formation than porous and coarse surfaces [34, 35]. There are several theoretical mechanisms that may explain this statement:

1. The surface topography may influence the protein deposition, which in turn may alter the cell response [20].
2. Cells grown on rough surfaces are more spherical than cells grown on smooth surfaces [36, 37] and DNA synthesis has been shown to be influenced by the cell shape [38]. Thus, the interfacial cells are probably in different functional states on smooth as compared with textured surfaces and this may induce different patterns of cell secretion products.
3. Mechanical stability at the interface has been suggested to be important for the tissue response and organization in both hard [4] and soft tissue [33, 39]. It is conceivable that the interfacial mechanical stability is larger for a coarse surface compared with a smooth. Thus, it has been shown that ingrowth of fibroblasts and collagen fibres can be achieved at structured implant surfaces.

Chehroudi *et al.* [18, 19] showed, for example, that fibroblasts were inserted obliquely in 22 μm deep grooves, while they were aligned parallel to surfaces with grooves of 3 and 10 μm and formed a capsule. The thin capsule formation around the coarse implants in the present study is in concordance with a recent study by Picha and Drake [40], where fibrous capsule formation was reduced on silicone–rubber discs with pillar-like surface structure compared to smooth control surfaces. This was also the case in a study of Brohim *et al.* [41] where most capsule formation was seen at the relatively smoothest silicone surface.

There are reports showing both an increased and decreased number of inflammatory cells when comparing smooth and textured surfaces. Our findings of lower number of macrophages at the interface to rough surfaces, especially at early time points, are in concordance with previous findings of Batra *et al.* [35], showing that micropillared silicone implants had less cellular response as compared to smooth implants at one week as well as at six months. Similar behaviour was also seen when comparing subcutaneous implants of polyethylene and polysulfone [42]. However, this is in contrast to Hunt *et al.* [25] who observed that polyurethane implants with a relatively rough microstructure elicited a larger macrophage response at all time periods compared with smoother implants. Von Recum [17] revealed that an optimal surface texture for soft tissue implantation had pores of 1–2 μm and pore sizes above or below this range displayed the usual inflammatory response with granulous tissue capsule formation. These contradictory findings between various studies may reflect either material or surface structure specific properties between the studies.

The increased capsule thickness over time for polyethylene implants used in this study is similar to the result in a recent study by Zhang *et al.* [30]. In this study it was found that the capsule thickness increased over time for polyurethane implants, whereas it was unchanged for silicone implants. In other studies the foreign body capsule thickness at metallic and ceramic implants in soft tissues has been found to be stable over time. In a study by Johansson *et al.* [43], the capsule thickness was measured at alloyed and non-alloyed titanium implants with essentially similar surface topography. The capsule did not differ in thickness between six and 12 weeks for the two investigated implants, neither were there any major differences in the cell response between the two materials. A similar response with respect to capsule formation was observed when analysing metal and ceramic implants over a 52 week time period [44]. The thickness of the capsule was relatively stable over time. These differences in the kinetic tissue response may be due to differences in long term stability and release of solubles from polymers as compared to metals and ceramics.

With regard to cellular densities at different implantation times this study showed a general decrease in cell numbers over time, which is in accordance with previous reports [26, 45]. Further, this study showed small but significant differences in the soft tissue response to polyethylene implants with smooth or textured surfaces, where a coarse surface (surface irregularities between 10 and 50 μm) elicited lesser tissue reactions.

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