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# The effect of titanium debris on soft tissue response

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The tissue response to titanium fracture plates after 18 months implantation has been analysed quantitatively, and the distribution of different cell types compared to the distribution of tissue discolouration. A tissue response including macrophages, fibroblasts and T lymphocytes, many of which were activated(CD25), helper/inducer(CD4), or cytotoxic/suppressor(CD8). These cells were indicative of a chronic granulomatous reaction. There was no correlation between the presence of such cells and their distribution around the plates with and without the presence and distribution of black debris in the tissue.

## 1. Introduction

Titanium and titanium alloys are used extensively in implanted devices. The extent of this can be seen in the biomaterials literature worldwide: 77 papers presented at the World Biomaterials Congress in Berlin April 1992 were related to titanium and its alloys and methods to improve or assess their biocompatibility. The effect of titanium wear debris has become particularly important with respect to the performance of orthopaedic devices made from commercially pure titanium or from a titanium-based alloy [1, 2]. There is some debate as to whether titanium debris is a primary cause of implant loosening or a secondary effect [3]. It has been possible to accurately identify the cell types found associated with these types of implants using monoclonal antibodies [4–6], fibroblasts, macrophages, and T lymphocytes being the cell types found in the greatest numbers, and with an absence of B lymphocytes. There is some disagreement, however, as to the activation state of these observed cells, the number of observed cells and their possible role at the implant site.

In contrast to the adverse effects seen with titanium wear debris in arthroplasty, titanium itself has been shown to display excellent biocompatibility under other circumstances, notwithstanding the tissue discolouration often seen in association with implants. The relationship between discolouration and cellular response has not been effectively studied, however.

This study focuses on the soft tissue response to commercially pure titanium from an area not complicated with the complex device interactions of joint protheses. The soft tissue covering plates used for internal fixation of tibia fractures was analysed after routine removal of the plates $1\frac{1}{2}$ years after implantation. The technique developed to quantitatively analyse the response to materials implanted into rats [7] has been modified to study responses of human tissue and the distribution of the debris. A panel of monoclonal antibodies were used to stain for specific cell types and an automatic image analysis system was used to quantify the cell numbers as well as address the issue of the cell distribution with respect to the fixation plate itself and the visual black titanium debris deposition found within the soft tissue.

## 2. Materials and methods

Human tissue in contact with a commercially pure titanium fracture fixation plate [8] was obtained during the routine removal of fixation plates in different clinics after $1\frac{1}{2}$ years implantation. The removal was supervised by the A.O./ASIF research institute in Davos. Tissue samples from 21 patients were taken. The tissue was snap frozen in liquid nitrogen, embedded in cryo-m-bed (Bright, UK) then serial sections were cut from the tissues blocks (7 μm thick) using a cryostat microtome. An immunostaining protocol [9] was followed to mark and stain the serial sections for the following cell types and subsets: macrophage (CD68 and HAM56), fibroblasts (propyl 4-hydroxylase), T lymphocytes interleukin-2 receptor (CD25), neutrophils (neutrophil elastase): these antibodies were obtained from Dakopatts, Denmark. Additionally, T lymphocytes (CD2, CD4 and CD8) and B lymphocytes (CD20) were obtained from Serotec UK. Mast cells were stained using chloroacetate esterase staining kit obtained from Sigma UK.

Each individual section was then analysed using an automatic image analysis system [7], with routines written specifically to select cells stained with these antibodies at a magnification of $\times 200$. A different routine was necessary for each antibody. An additional routine was written to analyse the debris itself. The total number of cells present within an area was measured ($2.0 \times 10^6$ μm$^2$) and their position relative to the fixation plate and to any debris was calculated. The amount of "visible" debris and the position of this

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debris was measured, the position of the cells and debris being presented as a pattern of cell or debris distribution.

Statistical analysis of the data obtained was performed using SAS (SAS Institute USA) Ver 6.04. A modelling program [10] was used to test the patterns of cell distribution. This allowed the relative significance of the following parameters to be calculated with respect to the tissue response: the "visible" debris, the cell type, and the patient. Analysis of variance (ANOVA), Waller Duncan and Tukey tests were performed on the cell count data to test the significance of the magnitude of the cellular responses.

## 3. Results

Macrophages, fibroblasts, T lymphocytes and neutrophils were observed in all the sections. The results from the different patients were divided into two distinct groups, a group showing no microscopically detectable debris and a group showing detectable amounts of debris within the soft tissue. Fig 1 shows the means of the total cell counts within these two groups. Statistically there was no significant difference in the magnitude of the individual cellular responses between these two groups for any of the cell types. No B lymphocytes were observed in any of the samples. Clearly the most predominant cells were macrophages (1141 $\pm$ 480) and fibroblasts (930 $\pm$ 376), with a smaller population of T lymphocytes (392 $\pm$ 137), 51% of which were activated T lymphocytes(CD25), 81% were helper/inducer(CD4), and 16% cytotoxic/suppressor(CD8). There was also a small neutrophil and mast cell response in the samples. The macrophage and fibroblasts populations were significantly greater than T lymphocyte, neutrophil and mast cell responses ($Pr > F$ 0.0001). Although different numbers of cells were observed for the different T lymphocyte markers there was no significant difference between the CD2, CD4 and CD25 results, only CD8 was significantly different ($Pr > F$ 0.0001).

Modelling the patterns of cell distribution revealed that all data fit the gamma parametric test best (Figs 2 and 3). Testing the relative effect of the variables revealed that the cell type had the most significant effect on the changing patterns of cell distribution observed, followed by an effect due to the patient. Of least significance was the effect due to observable debris (Tables I and II).

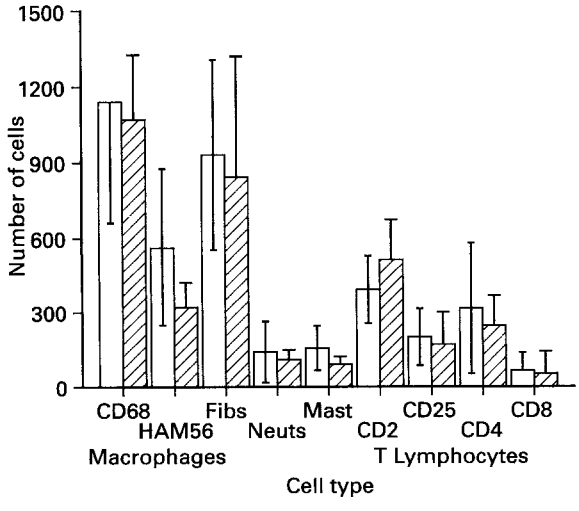


*Figure 1.* Means of total cell counts within the two patient groups (□ no debris, ▨ containing debris).

## 4. Discussion

The tissue response to this type of internal fixation device involved large numbers of fibroblasts and macrophages. The presence of the fibroblasts is accepted as being part of the normal wound healing response and their presence is expected around an implant material. The presence of large numbers of macrophages at $1\frac{1}{2}$ years after implantation indicates some form of chronic and sustained response due to this type of implant. There is likely to be constant release of debris from the implant surface due to the frictional forces between the tissue and the implant [11]. The presence of helper/inducer(CD4), suppressor/cytotoxic(CD8) T lymphocytes and a small number neutrophils, coupled with this large number of macrophages suggests that the observed tissue response is a chronic granulomatous inflammation reaction. The fact that a large proportion of the T lymphocytes are expressing IL2 receptors means this reaction is still occurring at this time, indicating that there is some continued stimulation from the implant or its products. From the presence of CD4 and CD8 positive cells a delayed hypersensitivity reaction cannot be excluded, the presence of mast cells adding to this hypothesis.

TABLE I Gamma log likelihood values for tested variables

| Model | Gamma |
|---|---|
| Deposit | 6517 |
| Patient | 6298 |
| Cell type | 5517 |
| Deposit block | 6250 |
| Deposit cell type | 5480 |
| Cell type block | 5214 |
| Deposit cell type block | 5180 |

TABLE II Model improvement tests: statistical significance of modelling from gamma log likelihood values

| Model | $-2(LL_1 - -LL_2)$ | $DF$ | $Pr = 0.0001$ |
|---|---|---|---|
| $C = DX$ v $C = DB$ | 1562 | 20 | Y |
| $C = DX$ v $C = DZ$ | 2000 | 6 | Y |
| $C = DZ$ v $C = DB$ | 438 | 20 | Y |
| $C = DBX$ v $C = DBZ$ | 2072 | 21 | Y |
| $C = DXZ$ v $C = DXB$ | 532 | 27 | Y |
| $C = DXZ$ v $C = DBZ$ | 1540 | 22 | Y |
| $C = DX$ v $C = DXZ$ | 74 | 8 | Y |
| $C = DX$ v $C = DXB$ | 606 | 27 | Y |
| $C = DXB$ v $C = DXBZ$ | 68 | 27 | Y |

$C$: Cell Count $D$: Distance
$X$: Cell Type $B$: Patient
$Z$: Deposit
$LL$ = Log likelihood.

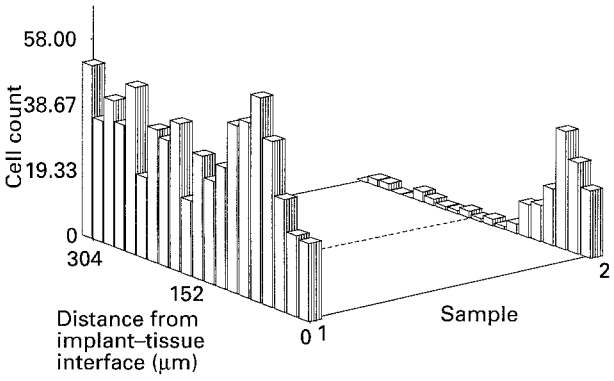


*Figure 2* Fibroblast distribution around titanium deposits (sample 1 = fibroblasts, sample 2. = deposits)

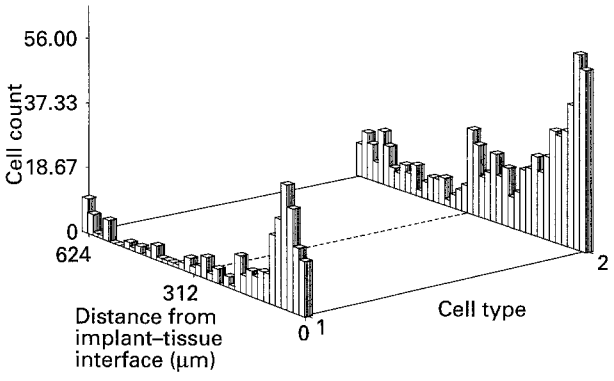


*Figure 3* Neutrophil and fibroblast distribution around titanium plate (cell type 1 = neutrophil; cell type 2 = fibroblast).

The visibly observable quantities of debris did not, however, appear to be the focus of this tissue response (Fig. 2), demonstrated by the pattern testing models, the presence or absence of visible tissue discolouration having the smallest effect on the pattern of cell distribution. As with other implanted materials the focus of the response is at the tissue implant interface (Fig 3). It is appreciated that titanium debris below 1 μm will be present in the tissue and will be unresolvable using light microscopy; those patients that did not demonstrate visible amounts of titanium will almost certainly have some debris in their soft tissue. It is relevant to divide the samples into these two groups as research has shown correlation between the amount of debris observed microscopically and the amount that is actually present [12], those patients with observable quantities of debris containing the most titanium/g of tissue. As titanium debris may constantly be released from the surface of the plate by abrasion with the soft tissue or by some corrosion process it is perhaps not surprising that the focus of the response is at this interface. However, it is surprising that the debris particles seem to pass through a phase of being a potent stimulator, then clump together into visible black groups and become quite passive again. It is perhaps not the quantity of debris that is important but the size of the debris particles. If the debris is predominantly very small the greater the likelihood of phagocytosis and therefore a more serious tissue response [3]. At very small particle sizes it is not so important what the particles are made of; their presence alone will stimulate a response. Goldring *et al.* [13] demonstrated that 1–10 μm sized debris were a strong stimulator of macrophages *in vitro*.

## 5. Conclusions

A tissue response involving fibroblasts, macrophages and T lymphocytes is observed in the soft tissue around titanium fracture fixation plates 18 months after implantation. This is consistent with a chronic granulomatous inflammatory reaction; a delayed hypersensitivity reaction cannot be excluded, however. The visible black debris sometimes observed in the soft tissue around such titanium implants is not the focus of the reaction.

## References


1. A V. LOMBERADI, T. H. MALLORY, B. K. VAUGHAN and P. DROUILLARD, *J. Bone Joint Surg.* [*Amer*]. **71-A** (1989) 1337.
2. M W. ELVES, J N. WILSON, J. T. SCALES and H B. S. KEMP, *Brit Med. J.* **4** (1975) 376.
3. R. L. BULY, M. H. HUO, E. SALVATI, W BRIEN and M. BANSAL, *J. Arthro* **7** (1992) 315.
4. P A. LALOR, P. A REVELL A B GRAY, S. WRIGHT, G T. RAILTON and M. A R FREEMAN, *J. Bone Joint Surg.* [*Brit*] **73-B** (1991) 25.
5. L. R. LINDBERG, O JOHNELL and L. LINDER, *Biomaterials* **9** (1988) 547.
6. S. SANTAVIRTA, Y T. KONTTINEN, V. HOIKKA and A ESKOLA, *J. Bone Joint Surg.* [*Brit*] **73-B** (1991) 38.
7. J. A HUNT, D. G. VINCE and D. F. WILLIAMS, *J. Biomed. Eng.* **15** (1993) 39
8. S. M. PERREN, K. KLAVE, O. POHLA, M PREDIER, S. STEINEMANN and E. GAUTIER, *Arch. Orthop. Trauma Surg.* **109** (1990) 304.
9. D G VINCE, J. A. HUNT and D. F. WILLIAMS, *Biomaterials* **12** (1991) 731.
10. J A HUNT, K R ABRAMS and D. F. WILLIAMS, *Math. Biol.*, in press.
11. P A LILLEY, D. R. MAY, P. S. WALKER and G. W. BLUNN, "Biomaterial Tissue Literations. Advances in Biomaterials", Vol. 10, edited by P. J. Doherty (Elsevier, 1992) p. 153.
12. H J. AGINS, N. W. ALCOCK, M BANSAL, E. A. SALVATI, P. D. WILSON, P. M PELLICCI and P. G. BULLOUGH *J. Bone Joint Surg.* **70-A** (1988) 347.
13. S. R. GOLDRING, N E. BENNETT, M. J JASTY and J. T. WANG, Particulate Debris from Medical Implants **1144** (1992) 136.