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The interaction between MDF cements and tissues

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A new high strength cement, developed with minimal internal flaws, has been investigated for use in surgery and dentistry. Preliminary results on the biocompatibility of this Macro-Defect-Free cement are given in this paper. The evidence suggests that the cement reacts slightly with the tissue environment, yielding some particulate debris. This increases the cellular activity and extends the period of the cellular inflammatory response following implantation.

1. Introduction

Within the last five years there have been significant developments in the preparation of hydraulic cements that allow them to achieve far better mechanical properties than are traditionally associated with such materials.

In particular, a series of cements referred to as Macro-Defect-Free (MDF) cements have been designed at ICI Mond Division. These make use of the intrinsic strength of some calcium-based substances by inhibiting the crack nucleation and propagation mechanisms that usually arise from the conventional fabrication techniques used in the preparation of cements [1, 2]. The development of mechanically strong cements that can be readily shaped into useful objects has opened up new and interesting possibilities for this generally unattractive type of material; indeed they have been described as "inorganic plastics" [3].

One possible area for exploitation of these new materials is that of surgery and dentistry where their match of elastic modulus with bone offers some advantage. Certain cements are already used for the lining of cavities in restorative dentistry, as "adhesives" for crown and bridge preparations and in some experimental surgical applications [4, 5]. The preliminary experiments described in this report were designed to assess the biocompatibility of such cements, by implantation of samples in the muscles of rats, as the first step in determining their suitability for surgical applications.

2. Materials and methods

One hundred grams of calcium aluminate cement (Secar 71, Lafarge) was mixed with 11 g of water and 7 g of polyvinyl alcohol/acetate (KH 17S, Nippon Gohsei), and rolled to form a sheet of dough which was pressed between Melinex films at room temperature for 30 min under 5 MPa pressure to give a uniform 1 mm thick plate. Discs 5 mm in diameter were punched from this sheet and the discs cured and dried between wooden sheets at 80° C for 24 h. Twelve of these disc-shaped samples were used in the study. Six samples were cleaned ultrasonically, dried and weighed ready for implantation. The other six were cleaned in a plasma ashing unit prior to weighing. Sterilization was achieved by autoclaving at 121° C (15 psi) for 15 min. Six black and white hooded Lister rats of the Liverpool strain were used. They were anaesthetized using the neuroleptanalgesic Immobilon. The lower part of the dorsal surface was shaved and thoroughly cleaned with alcohol. The samples were placed intra-muscularly into the para-spinal musculature and the wounds closed with polyglycolic acid sutures in the muscle and silk sutures in the skin. An ultrasonically cleaned sample was placed on the left side of the spinal column and a plasma

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ashed sample on the right. The animals were maintained on standard laboratory diet and water for the duration of the experiment. Two of the animals were sacrificed at four weeks, using chloroform, and the muscular tissues containing the implants were excised. The remaining animals were sacrificed at 9 and 30 weeks. With the 4- and 9-week samples, the tissues were immediately frozen on excision so that cryostat sections could be obtained for light microscopy. In addition the implants were carefully removed from the tissue and processed for scanning electron microscopy. The 30-week samples were prepared for both cryostat section and wax section histological examination.

For the frozen sections, the samples were excised from the tissue on removal from the animal. The pieces of tissue adjacent to and containing the implant site were secured between two metal discs (to give better heat conduction) and perfused with 10% sucrose. They were then quenched in liquid nitrogen-cooled isopentane and transferred to a cryostat, where 7 μm sections were cut at -15° C. The sections were then stained with haematoxylin and eosin. For the wax sections, conventional histological techniques were used involving prefixation of the tissue in formol saline, dehydration, wax embedding and sectioning at 5 μm on a microtome at room temperature, before haematoxylin and eosin staining.

Scanning electron microscopy was performed on the implant specimens with adherent tissue following glutaraldehyde fixation, dehydration, critical point drying and gold palladium coating [6]. A JEM35C scanning electron microscope was used at 15 kV.

In order to assess any changes taking place in the material on implantation, the same disc specimens from the 9-week period were compared with control samples in a diametral tensile test. Four control and four test samples were tested, using an Instron machine to load the axis across a diameter, with a cross-head speed of 1 mm min^{-1}. Failure was determined at the load F at which a crack spread along the diameter, and the diametral tensile strength σ calculated as

$$\sigma = \frac{2F}{\pi Dt}$$

where D and t are the diameter and thickness of the discs respectively.

3. Results

At four weeks the histology showed extensive infiltration of the muscle near to the implant sites. Numerous lymphocytes, including plasma cells, were evident. Macrophages were also present. The implant sites themselves were discrete, cell-filled capsules, easily discernible from the surrounding muscle. The entire capsular regions were bounded by layers of collagen, richly populated by fibroblasts. Large haemosiderin-laden macrophages were present, and a number of scattered lipid droplets could be seen. Some of the small muscle fibres close to the implant site had centrally located nuclei. Histologically there were no differences between left and right sides. Representative histological appearances are given in Fig. 1.

Scanning electron micrographs at 4 weeks showed little evidence of any interfacial reaction. A low power micrograph of one of the specimens is shown in Fig. 2a. There is clearly a thin layer of protein adherent to the surface, as one would expect. Evidence of cellular adhesion was present, in one case (Fig. 2b) with well preserved red blood cells; in another case (Fig. 2c) the denatured remnants of similar cells were present. In most specimens there was an unusual arrangement of circular patches of protein deposition, the reasons for this being unclear.

At nine weeks, histologically the reaction was very similar to that seen at 4 weeks, indicated in Fig. 3. The capsular area was still extremely cellular in nature, with many lymphocytes, macrophages and fibroblasts present, bounded by a narrow collagenous layer. This was a little unusual for, if the implants had been totally inert, the response of the tissue to the trauma of insertion should have subsided and the tissue would have been far less celluar.

Observations in the scanning electron microscope did not reveal any unusually cellular activity right at the interface, but did show that some interfacial reaction was taking place, yielding small clusters of reaction product of characteristic morphology, typically 5 μm in diameter (Fig. 4a). In some cases these deposits appeared covered in a protein layer (Fig. 4b). These deposits are analogous to those seen on the surface of some corrodable metals such as cobalt [6] and it is suggested that they represent a small degree of degradation of the cement within the tissue. It is not impossible that these clusters are merely tissue

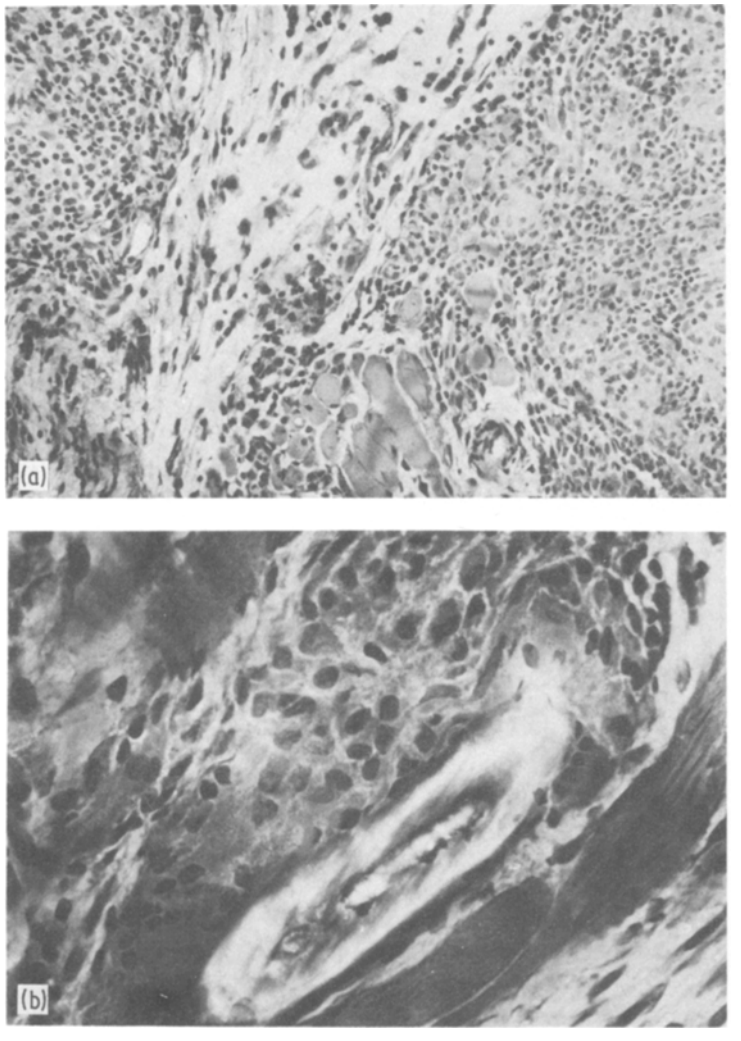


Figure 1 Representative histological sections at 4 weeks, showing extensive cellular infiltration.

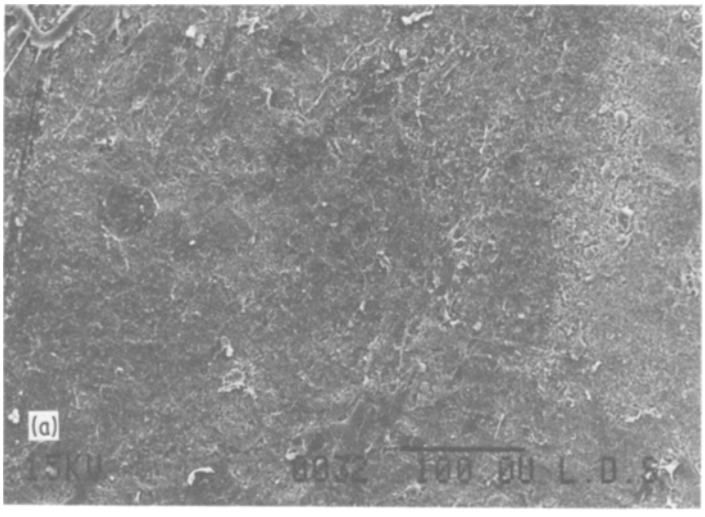
deposits, but experience with the scanning electron microscopy of other materials, where such deposits are not seen in the absence of corrosion or degradation, would suggest that degradation is occurring here.

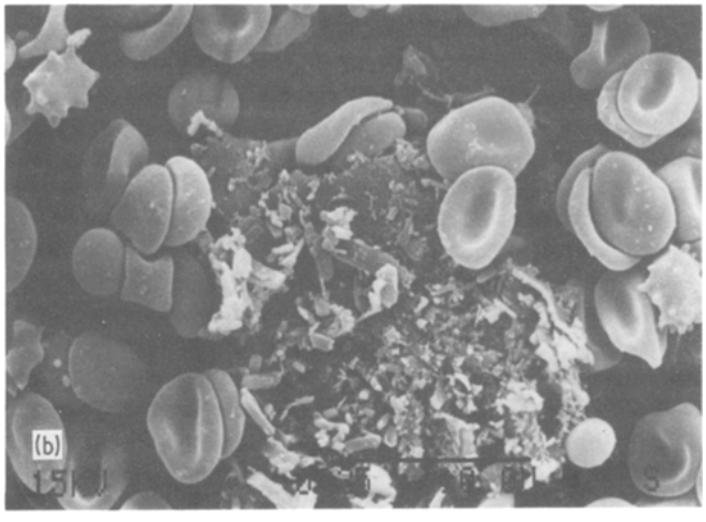
The histological appearance at 30 weeks showed that collagen deposition had occurred at the sites of implantation and a large fibroblastic population had been maintained within this area (Fig. 5). Capillary networks, in addition to larger vessels, were present. A few nervous elements were also apparent. The muscle near the implant seemed normal, although a number of muscle fibre islands were discernible among the collagen layers. At this time relatively few lymphocytes were present.

Deposits of a particulate material were noticeable in the sections at 30 weeks, as shown in Fig. 5. These particles exhibited strong bi-refringence under polarized light. Their morphology and appearance suggested that they were cement fragments. The only possible alternative is that the particles were remnants of the absorbable suture material used for muscle closure, but neither size or morphology were entirely consistent with the appearance of polyglycolic acid at 30 weeks post-implantation. A few macrophagic cell aggregates could be seen in close association with these deposits, but these were not extensive in nature.

Throughout the experiments the rats were healthy and showed no signs of abnormality. As a group they all gained weight as appropriate for rats of their age having undergone surgery. There was a mean net gain of 7 g at 4 weeks, 47 g at 9 weeks and 75 g at 30 weeks.

The results of the diametral tensile strength





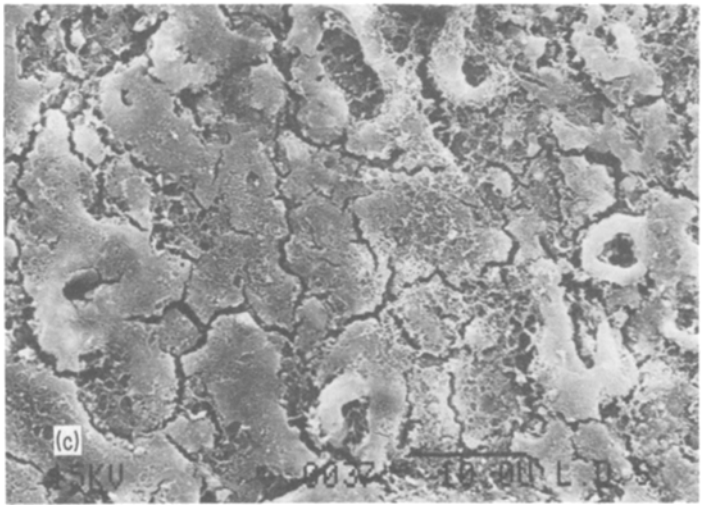


Figure 2 (a) Scanning electron micrographs of implant surface at 4 weeks, showing protein adherent to the cement. (b) Higher power scanning electron micrograph at 4 weeks showing good cell preservation. (c) As (b) showing denatured cells.

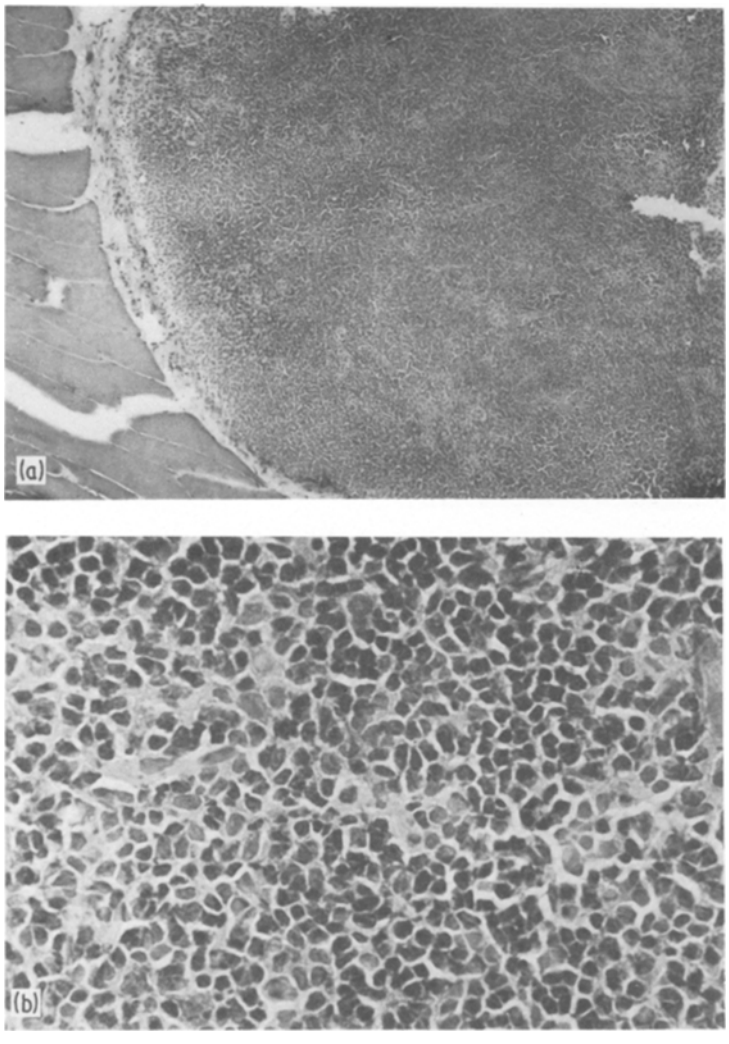


Figure 3 Representative histological sections at 9 weeks, showing persistent cellular infiltration.

tests showed the tensile strength σ of the controls to be 63 ± 4 MPa and that of the 9 week specimens to be 64 ± 4 MPa. The different tissue processing techniques used at 30 weeks prevented mechanical analysis of these specimens.

Attempts were made to assess specimen weight changes during the period of implantation. This proved difficult, however, as some tissue remained firmly adhered to the sample after removal from the tissue. Overall the cement samples gained weight during the period of implantation but the final weight changes probably represent a balance between a small amount of hydration and a smaller amount of material loss. At four weeks the net weight gain was 0.0022 g, (4%) at five weeks, 0.0028 g (6%) and at 30 weeks 0.0031 g (7%).

4. Discussion

The results and observations given above need to be considered in the context of the general response of tissue to the intramuscular implantation of synthetic materials such as metals [7]. This response will naturally vary with the nature of the material but can be related to the reaction of the tissue to the trauma of implantation. The incision into the muscle itself will provoke a well-defined sequence of events, broadly separated into inflammation and repair phases. During the inflammation phase, which is activated by the trauma and which involves the same characteristics whatever the origin of the injury, various cells invade the damaged region, where they attempt to deal with any persistent injurious agent and clear up damaged and necrotic tissue debris. Initially the cells will predominantly be polymorphonuclear leukocytes but many other cells may be present later, depending on the extent of the injury. Following on from, but also over-

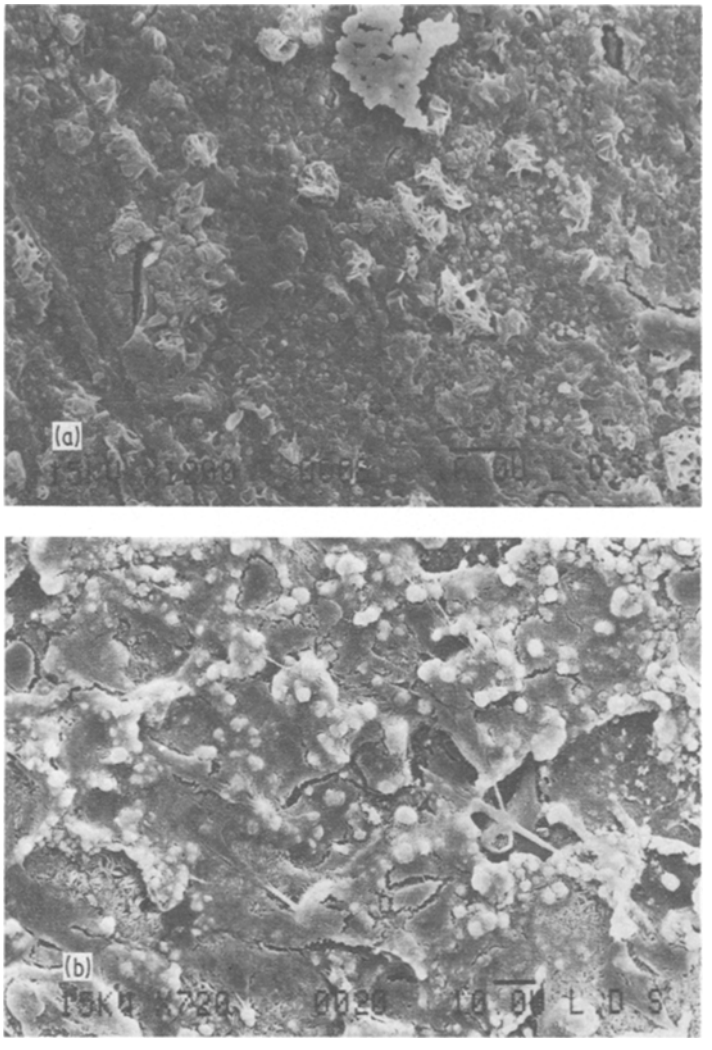


Figure 4 Scanning electron micrographs at 9 weeks showing reaction products on surface (a) and deposits partly covered with protein (b).

lapping the inflammatory phase to a certain extent, is the repair phase when fibroblasts synthesize and lay down new collagen to effect the reconstruction of the tissue. Very few tissues have the capability of regeneration in their own right and a zone of reparative fibrous tissue derived from this new collagen, known as scar tissue, will mark the site of the injury with a clean incisional wound. This scar tissue will be minimal and often difficult to find.

If there is some persistent injurious agent, then this response is modified. Specifically in this context, an implanted material can act as such an agent. If the material is chemically inert the modification of this response will be minimal. The end result of inflammation and repair will then be the formation around the implant of a capsule of fibrous, collagenous tissue of minimal thickness. With an increasing degree of chemical interaction, however, greater numbers and types of cell will infiltrate the area giving a more obviously modified response. While it is not possible to generalize completely the phenomenon and describe how different cell types respond to increasing irritation, a few observations can be made.

Firstly, if there is some chemical reaction between the implant and the tissue (for example, metallic corrosion or leaching of additives from polymers) then the predominantly polymorphonuclear response may give way to a macrophage and foreign body giant cell response. Secondly, if there is any antigenicity associated with the implant, cells of the immune response will be involved. Thirdly, if the interaction is continual, the response will be prolonged and the resolution

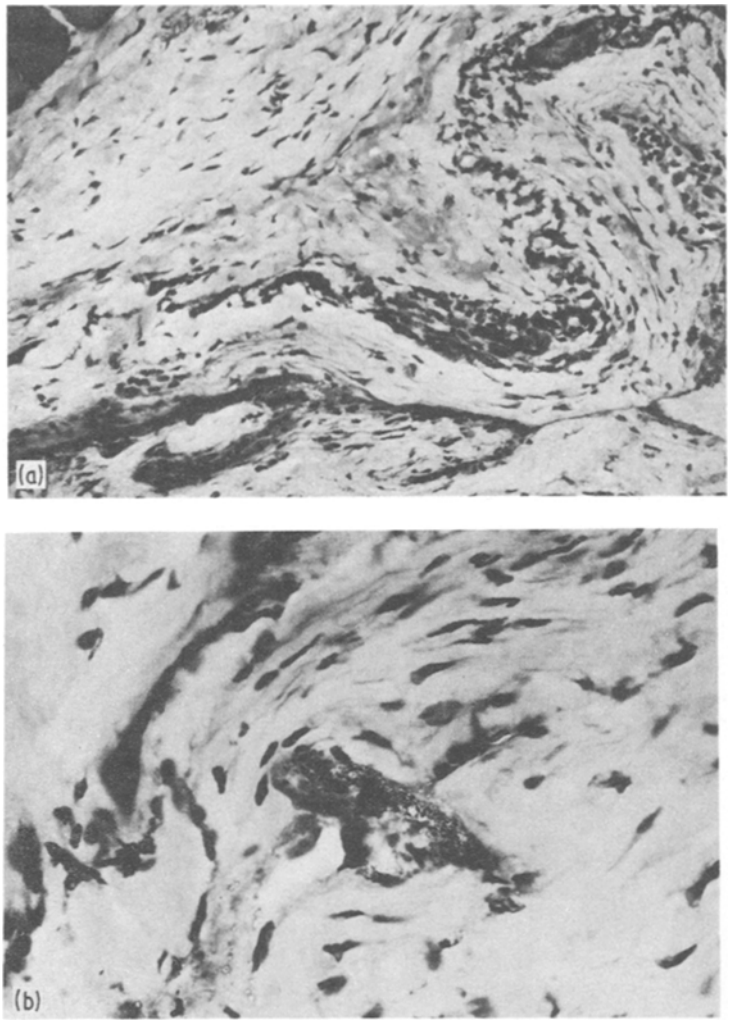


Figure 5 (a) Histological appearance at 30 weeks showing progress towards resolution of the tissue response. (b) Histological section at 30 weeks showing particulate debris in tissue.

of the repair process as described earlier will not take place in the normal short time. Specifically, with an inert material such as PTFE or alumina there should be a considerable decrease in the cellular activity associated with the response by 4 to 8 weeks. If there is a greater degree of irritation, the time scale is extended and cellular activity would still be obvious at this time. In severe cases the response would be indefinite and could result in considerable tissue necrosis. In less severe cases, it may take several months to achieve a stable situation, when the fibrous capsule will normally be thicker then with the inert material.

The situation with the MDF cement is that it appears to be slightly irritant. At four weeks the cellular reaction was evident but unremarkable. Had the material been inert this cellular activity would have largely subsided by nine weeks. This was not the case, however, for the area was still highly cellular with lymphocytes and macrophages in addition to the fibroblasts expected from the repair process. The scanning electron micrographs support the view that some interaction is taking place since small clusters of some reaction product could be discerned on the implant surfaces at 9 weeks.

This reaction, and the tissue response, cannot be described as severe since histologically the lymphocytes and macrophages had largely disappeared by 30 weeks leaving a large fibroblast population. There was still evidence of some degradation, however, as a few foreign particles were observed in the tissue, although these did not appear to be especially irritant. Generally the appearance at 30 weeks was suggestive of a slow resolution to repair. It would appear, therefore, that the material is not entirely inert but interacts

to a limited extent with the tissue environment, leading to a slightly prolonged cellular response.

These observations are consistent with the known behaviour of this MDF cement in aqueous environments. MDF cement samples, when immersed in water, undergo several changes as a result of moisture permeating into the pores. About four per cent by weight of water soaks in over a period of weeks, further reaction occurs with the calcium aluminate residues, and small amounts of hydrate reaction product appear at the surface.

Further experiments are underway with more stable versions of the cement to determine whether the tissue response is less cellular and resolves at a faster rate.

5. Conclusions

Samples of MDF cement have been tested for biocompatibility by intramuscular implantation in the rat and examination of the tissue response. The evidence indicates that the cement reacts slightly with the tissue environment yielding some particulate debris, although the strength of the cement remained unchanged. This increases the cellular activity and extends the period of the cellular inflammatory response to the implant. The response at 30 weeks, however, suggests that the effects of this interaction are only slight and that the response subsides appreciably, with the formation of a fibrous capsule and minimal necrosis. The observations would suggest that a cement with slightly less reactivity would evoke a very acceptable tissue response. Such experiments are now being undertaken.

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