THE INFLUENCE OF THE SURFACE ENERGY AND ROUGHNESS OF IMPLANTS ON BONE RESORPTION

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We investigated the possibility that the macrophages which are seen around implants may stimulate bone resorption and cause loosening. We found that macrophages release mediators that stimulate bone resorption, and that the amount of resorption increased by between 2.5 and 10 times when the macrophages adhered to a foreign surface. This bone resorption depended on the surface energy and roughness of the foreign surface, varying with these physical properties rather than with the chemical nature of the material. It is concluded that loosening of orthopaedic implants is likely to be influenced by the surface energy and roughness of the implant.

The most frequent and important long-term complication of total joint replacement is loosening of the components. Loosening will occur if the materials fail or if the surrounding bone is resorbed. Any foreign material, however inert, will stimulate a chronic inflammatory response known as the foreign body reaction (Coleman, King and Andrade 1974). A very marked foreign body reaction such as occurs when there is a large amount of wear debris from polytetrafluorethylene (PTFE) or high density polyethylene (HDP) is known to cause bone resorption (Charnley 1963; Revell et al 1978). It is therefore possible that the foreign body reaction to an implant, even in the absence of particulate debris, may stimulate bone resorption and hence cause loosening.

Of the cells involved in the foreign body reaction, macrophages and foreign body giant cells are the most likely to be responsible for bone resorption (Goldring et al 1986). Although these cells and osteoclasts are derived from the same stem cells and have many similar properties, neither macrophages nor foreign body giant cells are able to resorb bone in the manner of osteoclasts (Chambers and Horton 1984). There are numerous

mediators, for example prostaglandin E₂ (PGE₂), that both stimulate bone resorption and are released in inflammatory situations by macrophages and probably also by foreign body giant cells (Vaes 1988; Nathan 1987). So if bone resorption does result from the foreign body reaction it is likely to be stimulated by mediators, like PGE₂, that are released by macrophages or their multinucleate derivatives.

Macrophage migration and spreading has been shown to be influenced by the surface energy and roughness of the material on which they are cultured (Leake, Wright and Gristina 1981; Rich and Harris 1981). It is therefore possible that the amount of bone resorption stimulated by macrophages will also be influenced by the physical surface properties of the materials to which they adhere. Should certain physical surface properties be found to have an advantageous effect then implants could be manufactured with these properties.

The aim of our investigation is to determine if macrophages adhering to foreign materials release mediators that stimulate bone resorption, and to determine if the surface properties of the materials influence the bone resorption.

MATERIALS AND METHODS

Macrophages were cultured on various test materials. The medium in which they were cultured and into which they had released mediators was collected. This macrophage conditioned medium was assayed to determine both its effect on bone resorption, using a modified version of the neonatal mouse calvaria bone resorption assay (Reynolds 1976), and its PGE₂ content.

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Fig. 1

The contact angle method of assessing surface energy.

Macrophages. Macrophages were harvested from Tuck TO mice (A. Tuck and Sons, UK) by peritoneal lavage, using Ca and Mg free Hanks solution with 10% fetal bovine serum and heparin 5 U/ml. The peritoneal cells were washed, resuspended in Medium 199 with Earle's salts at a concentration of 10^6 cells/ml, and plated out at a density of 10^6 cells/cm² onto the test materials in 12-well tissue culture multi-dishes (Flow Labs, Rickmansworth). They were then cultured for 24 hours. (In the first experiment, to ensure that there was enough medium for the macrophages to be held in suspension, different culture conditions were used: the cell concentration was 7.5×10^5 cells/ml, the cell density was 2×10^6 cells/cm², and the culture duration was eight hours.) All media contained 1% antibiotics (penicillin 1 U/ml and streptomycin 5 mg/ml.)

Materials. In order to determine if the bone resorption depended on the material on which the macrophages were cultured, a series of orthopaedic materials were tested. Glass coverslips, the surfaces of which had been modified in various ways, were also tested so as to determine if the bone resorption was influenced by physical surface properties. Test materials were high density polyethylene (HDP), polytetrafluorethylene (PTFE), epoxy resin, polymethylmethacrylate (PMMA), stainless steel, and glass, all prepared as 22 mm diameter discs. These were ultrasonically cleaned in Decon, thoroughly rinsed, and then sterilised either by heat (180°C for four hours) or by immersion in 70% (v/v) ethanol. HDP discs were cut from an acetabular prosthesis (Chas. F. Thackray, Leeds), and stuck to glass coverslips by heat so that they would not float. Discs of PMMA, PTFE, and epoxy resin (matrix resin of Fibredux 920C, Ciba Geigy, Cambridge) were punched out of sheets. The sheets of PMMA were made by casting bone cement (CMW Labs Ltd, Clifton Road, Blackpool) between two glass slides. Medical grade stainless steel 316 was obtained in the form of polished discs. Untreated glass coverslips (Chance Propper Ltd, Smethwick, UK) were smooth; in order to obtain rough surfaces they were ground until opaque with 1 μ m aluminium oxide grinding powder (White Bauxalite 1200, Raymond A. Lamb, Acton, UK). Hydrophilic and hydrophobic surfaces were

obtained by treating the coverslips respectively by soaking in 4M potassium hydroxide, or by immersion in Sigmacote (Sigma, Poole, UK). Treated coverslips were thoroughly rinsed before being sterilised.

An assessment was made of the surface energy and roughness of all test materials. The contact angle between the surface of the test material and a drop of distilled water was used as a measure of surface energy (Fig. 1). A hydrophilic surface has a low contact angle and a high surface energy, and a hydrophobic surface has a contact angle of about 90° and a low surface energy. As it is not known which particular features of roughness are important one of the simplest methods of quantifying roughness was used: a surface that reflected an image was considered to be smooth and one that did not was considered to be rough.

Bone resorption assay. Calvaria were removed from four-day-old Tuck TO mice which had previously been injected subcutaneously with 1 μ Ci of ⁴⁵Ca. They were divided into halves, each containing a parietal and part of a frontal bone. This dissection was carried out in Medium 199 buffered with Hepes, under a dissecting microscope so as not to damage the periosteum. The calvaria halves were cultured for 24 hours in 1.5 ml of Medium 199 in 12-well multi-dishes on stainless steel grids, which kept them at the air-medium interface. They were then transferred onto fresh grids in fresh wells, and cultured in macrophage conditioned medium for a further 48 hours. The calvaria halves were used as pairs, with one half of each calvarium being cultured in medium conditioned by test macrophages and the other half in medium conditioned by control macrophages. The amounts of ⁴⁵Ca released into the medium during the 24-hour pre-incubation and the 48-hour incubation, and the amount remaining in the calvaria, which were dissolved in formic acid, were measured. The percentage of the total amount of ⁴⁵Ca that was released during the 48-hour incubation was determined, and used as a measure of bone resorption. A series of controls, negative (calvaria killed by freezing), unstimulated (fresh medium), and positive (PGE2 10^{-6} M) were also assayed.

Before the macrophage-conditioned medium was assayed it was serially diluted. At different doses different amounts of ⁴⁵Ca were released and a sigmoid dose-response curve was generated (means and standard errors of the mean (s.e.m.) were plotted). In order to determine if a specific treatment stimulated bone resorption significantly, the test and control ⁴⁵Ca release were compared at doses in the central portion of the dose-response curve, with a paired *t*-test. The magnitude of the bone resorption stimulus was determined from the amount the dose-response curve was shifted to the left. When a number of different treatments were compared one-way analysis of variance (ANOVA) was used.

Prostaglandin assay. The amount of PGE2 released by the macrophages was determined by radio-immune assay (New England Nuclear, Stevenage, UK). Medium

samples (100 μ l) were centrifuged at 1 000 rpm for 10 min, buffered (20 μ l of 0.5 M phosphate buffer), and then diluted to bring their PGE2 concentration to within the range of the assay. Tracer (100 μ l 125 I-labelled PGE2), antibody (100 μ l lyophilised rabbit anti-PGE2), and, following overnight incubation, cold precipitating reagent (1 ml) were added. After 25 minutes in ice the tubes were centrifuged at 1 500 rpm for 30 min at 4°C, and the radioactivity in the antibody-antigen complexes in the resultant pellet was measured using a gamma counter. A PGE2 standard curve (0.25 to 50 pg/100 μ l) was established for each assay and from this the sample PGE2 concentrations were calculated. Statistical analysis was by *t*-tests (non-paired).

RESULTS

Bone resorption stimulated by macrophage adherence. Two samples containing macrophages in suspension were cultured in siliconised glass flasks; in one flask the cells were allowed to adhere and in the other they were kept in suspension by a magnetic stirrer. The conditioned medium was assayed for bone resorption undiluted (Table I). It was found that macrophages in suspension release mediators that stimulate a highly significant amount of bone resorption. By comparing the 45 Ca release caused by macrophages in suspension with that caused by adherent macrophages it was found that macrophage adherence to a smooth hydrophobic surface also stimulated a highly significant ($p < 0.001$) amount of bone resorption (Table I). Using a dose-response curve it was calculated that the bone resorption stimulated by macrophages adherent to a smooth hydrophobic surface was 2.5 times that stimulated by macrophages in suspension.

Table I. Bone resorption stimulated by macrophage adherence

Sample	Number	45 Ca release (per cent)		
		Mean	S.e.m.	
Control (unstimulated)	12	9.36	0.39	
Macrophages in suspension	12	11.93	0.12	
Adhering macrophages	12	14.77	0.18	
Stimulus caused by macrophage adherence (paired)		2.84	0.17	$p < 0.001$

Bone resorption stimulated by different surfaces. In two experiments, macrophages were cultured on all the test materials, and the medium conditioned by the macrophages was assayed for bone resorption. It was found that different materials stimulated significantly different amounts of bone resorption ($p < 0.005$ in the first experiment and $p < 0.01$ in the second). In these and other experiments all pairs of materials were compared and the difference in 45 Ca release caused by each member of the pair was determined. The differences obtained

from all experiments were amalgamated (Table II). Based on these amalgamated differences the materials have been tabulated in the order in which they stimulate bone resorption (Table III). Also tabulated is their contact angle and roughness. In general the differences in bone resorption stimulated by adjacent materials in this order were not significant whereas the differences between materials separated by one or more places were significant.

Table II. The difference in bone resorption stimulated by macrophages adherent to different materials

	Stainless steel	PTFE	Epoxy resin	HDP	PMMA
Glass	0.59 NS	0.58 $p < 0.05$	1.23 $p < 0.001$	0.96 $p < 0.01$	1.84 $p < 0.001$
Stainless steel		0.34 NS	0.65 $p < 0.05$	0.86 $p < 0.001$	1.37 $p < 0.001$
PTFE			0.67 $p < 0.05$	0.41 NS	1.39 $p < 0.001$
Epoxy resin				0.08 NS	0.72 $p < 0.05$
HDP					0.82 $p < 0.001$

For each pair of materials the difference in 45 Ca release is tabulated, with the material named in the row causing more release than the one named in the column. The significance of each difference, determined by ANOVA, is based on the number of samples tested for each combination of material, which varied from 33 to 58. In these experiments glass had a hydrophilic smooth surface.

Table III. Bone resorption stimulation related to the contact angle, and the roughness of the test materials

Material	Bone resorption	Contact angle (degrees)	Roughness
Glass	Maximum	100	Smooth
Stainless steel	↓	20	Smooth
PTFE		100	Rough
Epoxy resin		70	Rough
HDP		90	Rough
PMMA	Minimum	60	Smooth

The effect of surface energy. Macrophages were cultured on hydrophilic and hydrophobic smooth glass surfaces. The contact angle of the former was 5° and of the latter 100°. The conditioned medium was serially diluted and assayed; the dose-response curves are shown in Figure 2. To confirm that the bone resorption was influenced by the surface energy this experiment (A) was repeated (experiment B) but only the intermediate dose was assayed. Using the calvaria as pairs the difference in 45 Ca release caused by the two surfaces was determined (Table IV). At each dose in the central portion of the dose-response curve the two surfaces caused significantly different 45 Ca releases ($p < 0.005$ and $p < 0.01$). From the shift of the dose-response curve it was calculated that the hydrophilic surface stimulated 2.5 times as much bone resorption as the hydrophobic surface.

Fig. 2

Bone resorption stimulated by macrophages adhering to surfaces of different energy.

Table IV. Bone resorption stimulated by macrophages cultured on surfaces of different energy. Calvaria used as pairs

Macrophage conditioned medium (Cells $\times$ days/ml)	Experiment	Difference in ^{45}Ca release (per cent) caused by hydrophilic and hydrophobic surfaces			
		Mean	S.e.m.	Number	Significance
10 $\times$ 10	A	-0.33	1.03	10	NS
2 $\times$ 10	A	2.35	0.96	8	$p < 0.05$
	B	2.96	1.23	10	NS
	Combined	2.69	0.79	18	$p < 0.005$
0.4 $\times$ 10	A	3.80	1.14	10	$p < 0.01$

The PGE₂ released by macrophages cultured on surfaces of different energy was determined in two separate experiments (see Table VI). In both it was significantly more on a hydrophilic surface than on a hydrophobic surface ($p < 0.05$ and $p < 0.001$), and on average the release on a hydrophilic surface was 1.5 times greater than on a hydrophobic surface.

The effect of roughness. Macrophages were cultured on rough and smooth hydrophilic glass surfaces. The conditioned medium was serially diluted and assayed; the dose-response curves are shown in Figure 3. This

Fig. 3

Bone resorption stimulated by macrophages adhering to surfaces of different roughness.

experiment (C) was repeated (D), and using the calvaria as pairs the differences in ^{45}Ca releases caused by the two surfaces was determined (Table V). At each concentration on the central part of the dose-response curve the rough surface stimulated significantly more release than the smooth surface ($p < 0.005$ and $p < 0.02$). The rough surface stimulated about twice as much bone resorption as the smooth.

The PGE2 released by macrophages cultured on surfaces of different roughness was determined in three experiments (Table VI). In all three more PGE2 was released on a rough surface than on a smooth surface, and in two the difference was significant ($p < 0.005$ and $p < 0.01$). On average the release on a rough surface was 1.4 times greater than on a smooth surface.

The combined influence of surface energy and roughness. In one experiment macrophages were cultured on rough and smooth hydrophilic, and rough and smooth hydrophobic glass surfaces, and the conditioned medium was assayed both for bone resorption and PGE2 (Table VI). It was found that rough hydrophilic surfaces stimulated the most bone resorption and PGE2 release and smooth hydrophobic the least. In between were the smooth hydrophilic and rough hydrophobic, with the former stimulating slightly more although the differences were not significant.

DISCUSSION

It has been demonstrated that macrophages release soluble factors which stimulate bone resorption, and that when they adhere to a foreign surface the amount of bone resorption they stimulate increases by a factor of at least 2.5. When a foreign material is implanted it becomes surrounded by a large number of macrophages which adhere to it. Implant loosening may therefore be initiated by bone resorption stimulated by adherent macrophages. It is important to determine if different orthopaedic materials stimulate different amounts of bone resorption and so will influence the chance of loosening.

We tested six orthopaedic materials and found that macrophages adhering to them stimulated significantly different amounts of bone resorption. To determine why different amounts of bone resorption were stimulated, we selected one material, glass, and investigated the effect of modifying its physical surface properties. We found that surface energy and surface roughness had a highly significant influence on both bone resorption and PGE2 release. A hydrophilic surface stimulated 2.5 times as much bone resorption and 1.5 times as much PGE2 release as a hydrophobic surface. A rough surface stimulated twice as much bone resorption and 1.4 times as much PGE2 release as a smooth surface. When we tested all four possible combinations we found that, regardless of the surface roughness, hydrophilic surfaces stimulated more bone resorption and PGE2 release than hydrophobic surfaces, although the differences were not

Table V. Bone resorption stimulated by macrophages cultured on surfaces of different roughness. Calvaria used as pairs

Macrophage conditioned medium		Difference in ^{45}Ca release (per cent) caused by hydrophilic and hydrophobic surfaces			
		Mean	S.e.m.	Number	Significance
Cells $\times$ days/ml	Experiment				
10×10^5	C	-0.51	0.54	10	NS
2×10^5	C	1.11	0.38	10	$p < 0.02$
	D	0.88	0.41	12	NS
	Combined	0.99	0.27	22	$p < 0.005$
0.4×10^5	C	2.22	0.77	10	$p < 0.02$
	D	0.50	0.49	12	NS
	Combined	1.28	0.47	22	$p < 0.02$

Table VI. PGE2 release by macrophages cultured on different surfaces ($\text{ng}/10^6$ cells.day)

Test surface		Control surface		Difference		
				Mean	Factor	Significance
Mean	S.e.m.	Mean	S.e.m.	Number		
Hydrophobic (smooth)		Hydrophilic (smooth)				
0.23	0.02	0.32	0.03	6	0.72	$p < 0.05$
0.68	0.06*	1.13	0.06*	4	0.60	$p < 0.001$
Rough (hydrophilic)		Smooth (hydrophilic)				
4.91	0.23	3.44	0.24	6	1.43	$p < 0.005$
11.32	1.16	7.10	0.26	6	1.59	$p < 0.01$
1.18	0.01*	1.13	0.06*	4	1.04	NS

*These results were all obtained from one experiment in which the PGE2 release caused by a rough hydrophobic surface was also assayed and was 0.95 (s.e.m. 0.05)

always significant. The fact that the PGE2 release paralleled the bone resorption stimulus makes the bone resorption results more significant. When a comparison was made between the bone resorption stimulus and the surface properties of the six test materials (Table III), it was again found that the materials with hydrophilic surfaces (glass and stainless steel) stimulated more bone resorption than those with hydrophobic surfaces (PTFE, epoxy resin, HDP and PMMA). It was also found that within the group having hydrophobic surfaces, those with rough surfaces (PTFE, epoxy resin and HDP) stimulated more bone resorption than did those with a smooth surface (PMMA). As the bone resorption correlated with the physical surface properties of a variety of different materials it is likely that the physical surface properties of implants are more important than their chemical nature.

The physical surface properties, in particular surface energy and surface roughness, of the materials to which macrophages adhere have been shown to influence the amount of bone resorption that they stimulate. The effect of these two surface properties is cumulative, and of the two surface energy is probably the more important. Although rough hydrophilic surfaces have been shown to stimulate approximately five times more bone resorption than smooth hydrophobic surfaces it cannot be concluded that smooth hydrophobic surfaces will function best

clinically. This is primarily because loosening will occur if bone resorption exceeds bone formation, or in other words, if there is net bone resorption. Bone formation is coupled to bone resorption and many mediators, such as PGE₂, that stimulate bone resorption also stimulate bone formation. Therefore if two surfaces are compared the one that stimulates most absolute bone resorption in vitro will not necessarily cause the most *net* resorption in vivo. The balance between formation and resorption produced by hydrophilic/rough surfaces and hydrophobic/smooth surfaces can only be determined by in vivo experiments.

Conclusions. We have shown that when macrophages adhere to implants they release mediators that stimulate local bone resorption. This is a possible mechanism by which implant loosening is initiated. The amount of bone resorption and hence the chance of loosening has been shown to be influenced by the surface energy and roughness of the implant. It has also been shown that these physical surface properties are more important than the chemical nature of the implant materials.

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