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A simple optical method for the differentiation of two types of polymeric wear debris in tissue samples

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A simple low-cost optical method for differentiating two birefringent plastic materials in tissue sections is described. The method relies on the measurement of the refractive indices of the materials as standard samples, then mounting the specimen containing the mixture of materials in a medium of intermediate refractive index. The optical properties of the materials in this mounting medium permit their separate identification by use of Becke's lines. In the specific example used, ultra-high molecular weight polyethylene (UHMWPE; refractive indices 1.521 and 1.529) and polyacetal (refractive indices 1.476 and 1.492) were distinguishable from each other by mounting in sandalwood oil (refractive index 1.510). Illustrative results are given for the analysis of the comparative amounts of these two polymers in the tissues adjacent to five knee replacements obtained at revision surgery. In every case there were more UHMWPE particles than polyacetal particles.

1. Introduction

Although the majority of prosthetic joint implants have components made in metal alloy and high-density polyethylene, other materials such as ceramics and other plastics are also used. Advances in materials science have led to the development of "second generation" polymers, some of which may find use in joint replacement surgery. When prosthetic joints fail, the histopathologist is faced with the problem of assessing the type of wear debris present in the tissues and the amounts of such debris. Such information is vital to the continuing evaluation of new materials and designs. Metal particles are easily detected histologically and can be differentiated by various forms of elemental analysis, such as electron-probe microanalysis, atomic absorption spectrophotometry or neutron activation analysis. Particles of plastic are readily identified by polarization light microscopy, since they are birefringent. Unfortunately, there is little to distinguish the appearances of one type of plastic from another by this method, and differentiation of polymeric materials depends on the use of sophisticated analytical techniques such as infrared spectroscopy and microscopy, which are themselves of limited availability and difficult to apply to the detection of particulate material deeply embedded in cellular tissue.

We have devised a simple optical method for detecting two different types of plastic particle and have demonstrated its usefulness in examining tissues from individuals undergoing revision arthroplasty. Here we report this method, which in theory could be applied to the differentiation of any two birefringent materials, and show its application with results obtained in a study of individual cases.

2. Materials and methods

The refractive index of a material is constant for that material for a given wavelength of light, and birefringent materials have two such indices in different axes. The method that we have developed depends on the accurate measurement of the refractive indices of the two birefringent materials under investigation, followed by the mounting of the sample containing them in an appropriate medium which will allow identification of the two different plastics using simple optical principles. The method necessitates the determination of the refractive index of known fluids, the preparation of standards and the use of these to measure the refractive indices of the birefringent materials.

2.1. Measurement of refractive index of standard liquids

An Abbe refractometer was used to determine the refractive index of a series of liquids. Briefly, each liquid was instilled into the narrow space between two optical prisms in the apparatus. Parallel rays of light were reflected into the prisms, where they were focused by a telescope to form a fine line. The telescope was then adjusted to make the line coincide with the intersections of cross-wires in the eyepiece. The angle was read on a graduated scale directly as the refractive index, calibrated from 1.300 to 1.700 in 0.001 intervals. The fourth decimal place was estimated, to give an accuracy of refractive index of 0.0002. The prisms in the refractometer were surrounded by a water jacket to control the temperature accurately. (The refractive index of a liquid varies with temperature, e.g. that of distilled water at 10 °C is 1.3337 and at 40 °C is

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1.3307.) The refractometer was calibrated each day by adjusting the scale setting and using distilled water at constant temperature (20 °C; refractive index 1.3330) as the standard.

The refractive indices of a series of liquids chosen to give a range of values between 1.3330 (distilled water) and 1.632 (monochloronaphthalene) were measured. Detailed results are given in Section 3. Liquids of intermediate refractive index were prepared with mixtures of these standard liquids using the equation

$$v_1 n_1 + v_2 n_2 = (v_1 + v_2) n_x$$

where v_1 and v_2 are the volumes of the standard liquids, n_1 and n_2 are their refractive indices and n_x is the required refractive index.

It was necessary to prepare fresh standard mixtures on each occasion and to check the refractive index before use, because of the effects of temperature both on the refractive index and on the loss through volatility of some liquids.

2.2. Measurement of refractive indices of birefringent materials

Small particles were prepared from blocks of ultra-high molecular weight polyethylene (UHMWPE) and polyacetal using a half-round rasp which produced shavings of the same size order as those seen in tissue sections of synovium containing polymeric debris at revision arthroplasty in man (approximately 50 µm × 5 µm). Samples of the shavings were placed on a microscope slide, immersed in one of the standard solutions prepared as above, then viewed by polarization and conventional light microscopy at 100 × and 250 × magnifications, the former to localize the particles, the latter to determine the refractive index by the Becke method. This can be described, briefly, as follows. The junction between an object and the surrounding liquid medium is focused sharply and the image is then deliberately thrown out of focus by raising the objective. A bright band of light appears at the junction and moves towards the substance of higher refractive index, becoming broader and fainter as it does so. Lowering the objective has the opposite effect, the bright line passing towards the substance of lower refractive index. A birefringent object will no longer show this phenomenon of a Becke line in one axis when the refractive index in this axis coincides with that of the surrounding liquid medium. It is therefore possible to obtain values for both of the refractive indices of an unknown birefringent polymer fragment by immersion in a series of different standard liquids.

2.3. Optical differentiation of two polymeric materials

Having determined the refractive indices of the two materials under investigation, in this case UHMWPE and polyacetal, samples were prepared and mounted in a medium of refractive index midway between those of the two polymers. Sandalwood oil (refractive index 1.510) was chosen for this purpose. The ability to differentiate between UHMWPE and polyacetal using the Becke method was checked using known samples prepared *in vitro* and mounted in sandalwood oil. Samples of UHMWPE alone, polyacetal alone and a mixture of UHMWPE and polyacetal were examined "blind" by one of us (S. O'S.). The recognition of samples was completely (100%) reliable and reproducible. The method was then applied to tissue sections from five cases in which UHMWPE and polyacetal were the component materials in Freeman knee joint replacements coming to revision arthroplasty. In each case the femoral component was made of polyacetal and the tibial component of UHMWPE. There were four females and one male, aged between 59 and 87 years, who had come to revision surgery 6, 13, 16, 24 and 31 months after initial arthroplasty. The samples were all from the synovium and 5 µm-thick paraffin wax-embedded sections were stained with toluidine blue after pretreatment with lyophilized trypsin (250 000 units) in 25 ml isotonic saline at 37 °C for 30 min, to remove any protein coating from the surface of the particles.

3. Results

3.1. Refractive index measurements

The refractive indices of the various standard liquids were determined using an Abbe refractometer and are shown in Table I. Mixtures of these liquids were used to prepare further samples of intermediate refractive index for the determinations of refractive indices of particulates, using the Becke method.

The refractive indices of UHMWPE were 1.521 and 1.529, and those of polyacetal were 1.476 and 1.491, as determined by the Becke method on abraded flakes of these materials using standard liquids of known refractive index.

3.2. Differentiation of UHMWPE and polyacetal

Shavings of UHMWPE and polyacetal appear almost invisible by ordinary light microscopy, but show up as brightly birefringent fragments when viewed by polarization microscopy. Polarization is therefore useful to identify the particles (Figs 1 and 2) before optical

TABLE I Refractive indices of a series of liquids determined by using an Abbe refractometer at 20 °C

Liquid	Refractive index at 20 °C
Distilled water (constant)	1.333
Ethyl alcohol	1.362
Olive oil	1.470
Turpentine	1.470
Castor oil	1.480
Toluene	1.498
m-Xylene	1.500
Sandalwood oil	1.510
Monobromobenzene	1.560
Bromoform	1.598
Monochloronaphthalene	1.632

analysis to differentiate between the two polymers. Viewed by conventional light microscopy, the Becke line shows up clearly as a thin, bright band of light at the edge of the polymer fragment immersed in sandalwood oil (refractive index 1.510). When the objective is raised from the position of sharpest focus, the Becke line moves into the fragment if the material has a higher refractive index (UHMWPE) than sandalwood oil (Fig. 3) and out from the fragment when the objective is lowered (Fig. 4). The material with a lower refractive index (polyacetal) than sandalwood oil shows the opposite effect, the Becke line moving into the fragment when the objective is lowered and out of the fragment when it is raised (Figs 5 and 6).

3.3. Examination of synovial tissues containing UHMWPE and polyacetal

Synovial tissue sections from five cases undergoing revision surgery for Freeman knee arthroplasties in which there were polyacetal and UHMWPE components were examined in sandalwood oil.

Two histological sections were examined from each tissue block in four of the cases, and two sites were

Figure 1 A shaving of UHMWPE, viewed with crossed polars in the light microscope, showing the birefringent nature of this material.

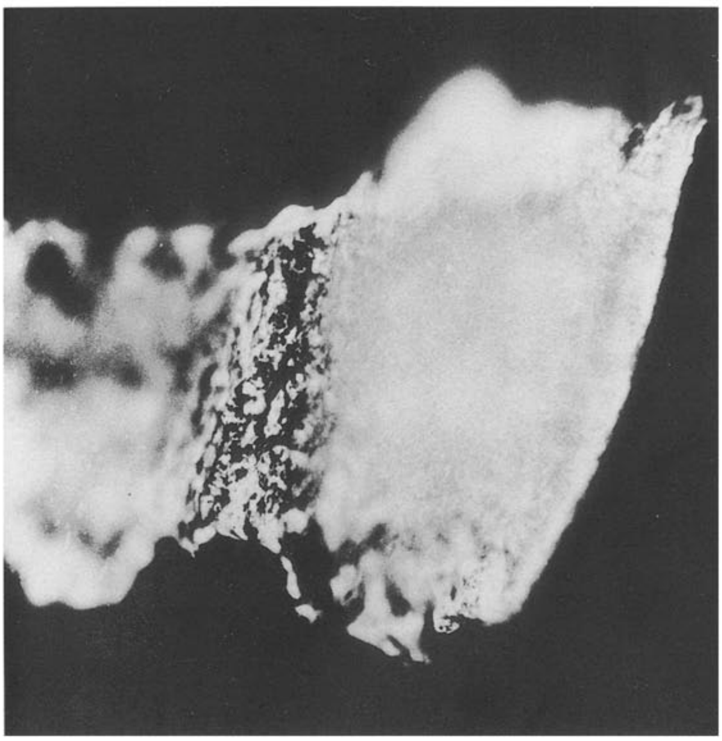

Figure 2 A shaving of polyacetal, viewed with crossed polars in the light microscope, showing the birefringent nature of this material. Distinction from UHMWPE by polarization microscopy is impossible (compare with Fig. 1).

compared in two cases (nos 1 and 2) and four levels of histological section from one site in the fifth case. Fifty particles were inspected in each histological section examined. The results are shown in Table II.

These results show that there is some variation from one site to another in any individual case, but that there is less variability in different sampling levels of tissue taken from the same site. In no case did polyacetal debris predominate over UHMWPE, and usually there was much more UHMWPE present in the tissues.

4. Discussion

A large majority of current designs of prosthetic implants in orthopaedic surgery use metal alloys articulating against UHMWPE, but the increasing development and use of polymers means that the question of differentiating between two such materials in tissue sections arises. There are few descriptions of attempts to solve this problem. Leugering and Puschner [1] used a method based on the determination of the melting point of a linear polyester (Hostadur KVP 4022) and partially crystalline acetal resin (Hostaform

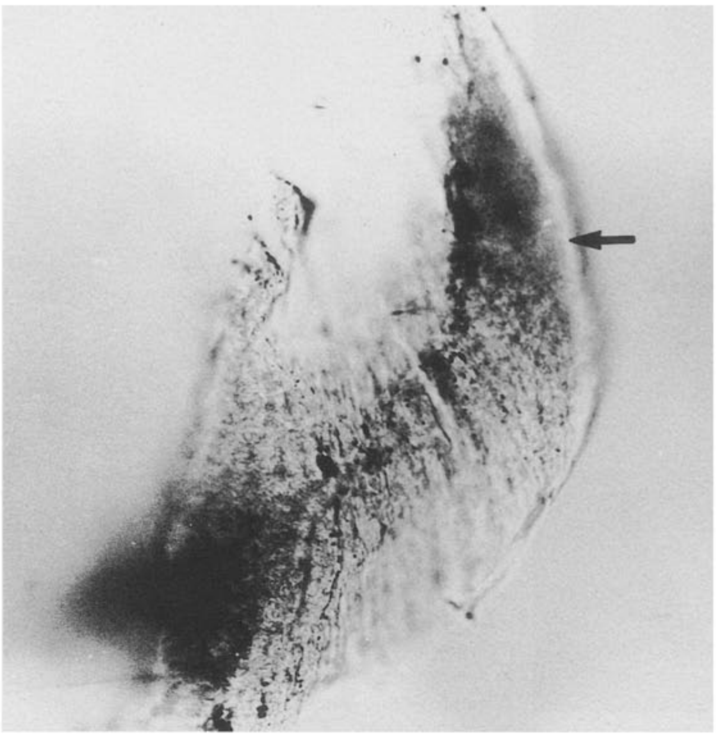

Figure 3 The same shaving of UHMWPE as in Fig. 1, viewed by conventional light microscopy. The objective of the microscope has been raised slightly from the position of sharpest focus. A bright line (Becke line) appears to have entered the shaving, indicated by the arrow.

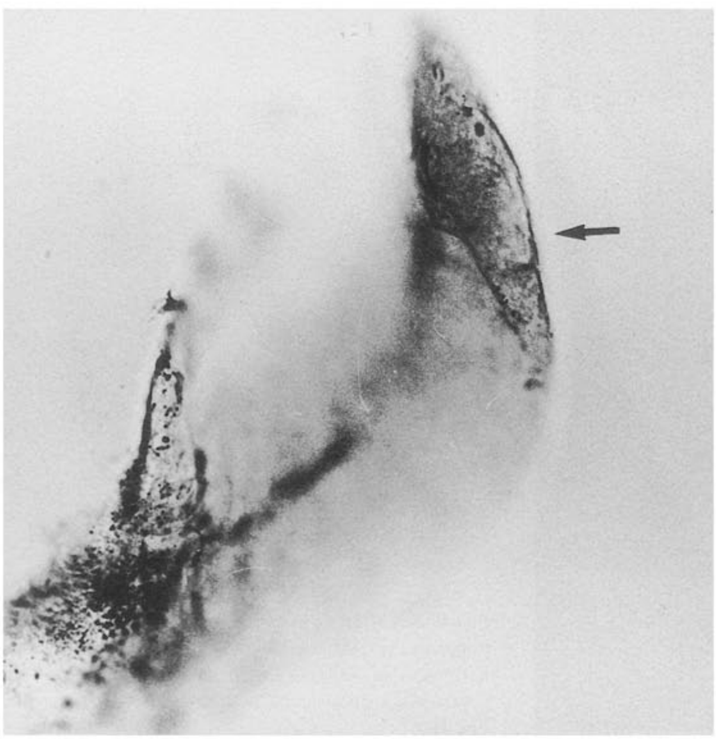

Figure 4 The same shaving of UHMWPE as in Fig. 1, viewed by conventional light microscopy, but with the objective lowered slightly from the position of sharpest focus. The Becke line (arrow) appears to have moved outwards from the true edge.

CZ 520) to distinguish these two polymers. In theory, infrared spectroscopy and microscopy could also be applied to the problem, but there are no accounts of their use in this way. Although the method we have described here was employed specifically to distinguish UHMWPE from polyacetal, we believe that it could be easily adapted to the differentiation of other particles, so long as they had sufficiently different refractive indices.

The method depends on the identification of a mounting medium of refractive index that is intermediate between the refractive indices of the two polymers to be recognized, and relies on the use of Becke lines visualized by conventional light microscopy. When a convergent beam of light is directed at the junction of two materials of different refractive index, rays passing from the material with the lower refractive index to that with the higher refractive index are bent towards the normal. Rays passing from the higher to the lower refractive index material follow a different pattern, since they impinge at less than the critical angle and are reflected without crossing the junction. This produces a more brightly illuminated area on the side of the material with the higher

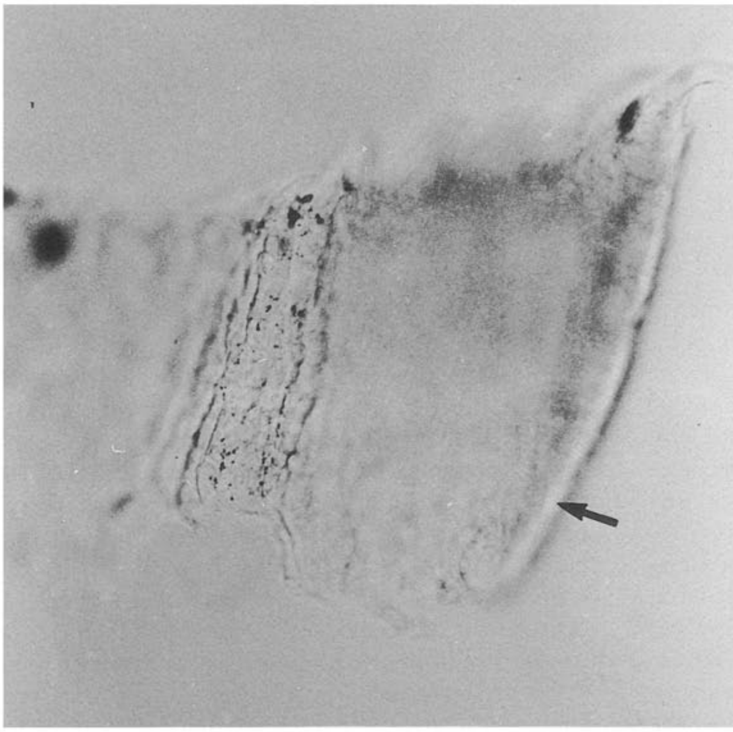

Figure 5 The same shaving of polyacetal as in Fig. 2, viewed by conventional light microscopy. The objective of the microscope has been lowered slightly from the position of sharpest focus. The bright Becke line appears to have moved inwards from the true edge, as indicated by the arrow. Compare with Fig. 4 (lowered objective, UHMWPE).

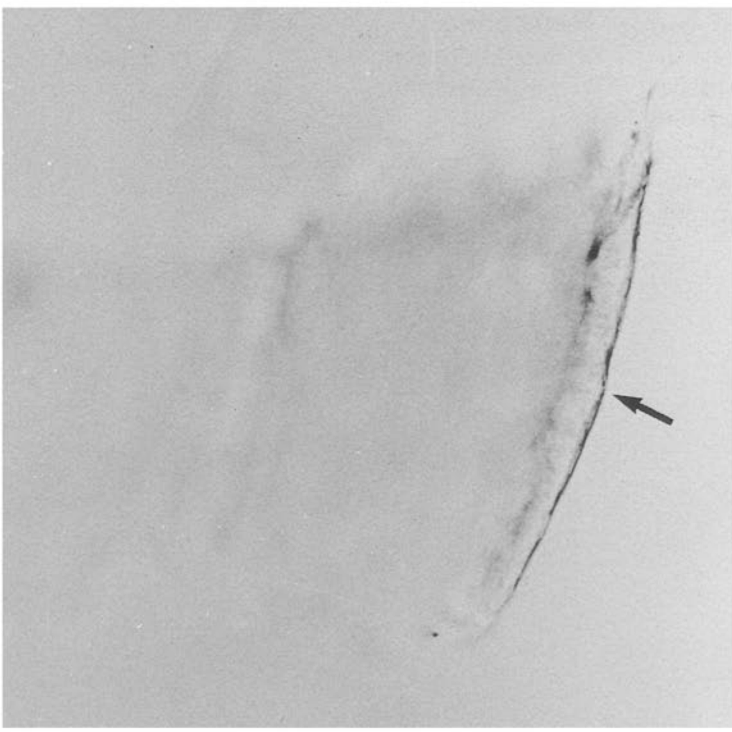

Figure 6 The same shaving of polyacetal as in Fig. 2, viewed by conventional light microscopy, but with the objective raised slightly from the position of sharpest focus. The Becke line (arrow) appears to have moved outwards from the true edge. Compare with Fig. 3 (raised objective, UHMWPE).

TABLE II Numbers and percentages of UHMWPE and polyacetal particles in synovial tissue samples from five cases undergoing revision surgery following Freeman knee arthroplasty

Case no. (section)	Number of UHMWPE particles	Number of polyacetal particles	Percentage UHMWPE particles	Percentage polyacetal particles
1A (1)	17	33	34	66
(2)	29	21	58	42
1B (1)	45	5	90	10
(2)	39	11	78	22
2A (1)	47	3	94	6
(2)	46	4	92	8
2B (1)	36	14	72	28
(2)	42	8	84	16
3 (1)	45	5	90	10
(2)	50	0	100	0
4 (1)	27	23	54	46
(2)	25	25	50	50
5 (1)	29	21	58	42
(2)	36	14	72	28
(3)	34	16	68	32
(4)	44	6	88	12

refractive index. A debris particle of higher refractive index than its surrounding medium acts rather like a biconvex lens with rays converging above it at the point of sharpest focus. A particle of lower refractive index than its surroundings acts as a biconcave lens. In the former case the interior of the object becomes more brightly illuminated as the objective is raised and the Becke line moves inwards.

It is not the object of this paper to present anything more than sample results using this method. We have, however, shown that it is possible to distinguish between UHMWPE and polyacetal in tissue sections. Under these circumstances we found it necessary to use a trypsin-digestion stage before visualizing the particle-containing samples. Without this stage clear recognition of the edge of the particles is difficult, and we believe that the digestion procedure may have resulted in clarification of the images of the particles by removal of any surface coating from the particles. Such a proteinaceous layer would have its own refractive index and therefore interfere with the optical mechanism which applies to the analysis of the interfaces between materials of predetermined refractive indices (viz. UHMWPE and polyacetal versus sandalwood oil).

It is not possible to state how much of the variation between the percentages of the different polymers detected in different samples is real or apparent. Blind examination of separately prepared samples of the two materials examined individually or as a mixture showed this to be a reliable method. The fact that there was some variability in the percentages of the different polymers even from the same site but in sections from different levels suggests that there is true variation. Within one particular case, however, there was broad agreement over which polymer was predominant.

Reference

1. H. J. LEUGERING and H. PUSCHNER, *J. Biomed. Mater. Res.* **12** (1978) 571.

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