

CHAPTER 5

RETRIEVAL STUDY

5.1 Introduction

Retrievals obtained after revision surgery provided valuable information with respect to the causes of wear that take place in-vivo.

Note: For better clarity, please view all photographs (jpg format) on the CD enclosed.

The components investigated were randomly retrieved from one centre, properly marked and the details of the patients were noted for further reference. In all, 57 components were retrieved. Forty-seven (47) components were brought to the laboratory for an engineering investigation into the causes of failure. The ISO standard for the removal and handling of retrievals was followed (ISO 12891-3, 2000). The bearing couples varied between steel on UHMWPE and ceramic on UHMWPE

A second investigation was conducted making use of a further 5 components retrieved during revision surgery. During this second study all the freshly retrieved components were analysed in a biochemistry laboratory within one hour after removal from the patient. The purpose of this investigation was to try and determine if any proteins were deposited inside the cup. These components were not cleaned in theatre but were supplied as retrieved. The findings of this investigation are discussed in paragraph 5.4.

A third investigation was conducted also making use of 10 freshly retrieved components. These retrievals also included tissue removed from the patients. The purpose of this investigation was to try to find wear debris on either the retrieved components or in the tissue surrounding the prosthesis in-vivo to enable the qualification of the mechanism creating this debris. The detail of this investigation will be discussed in paragraph 5.8.

During this investigation, the following techniques were used to inspect and analyse the retrieved components:

- a. Visual inspection
- b. Investigation making use of a colour dye penetrant
- c. Investigation under a stereo microscope
- d. Investigation making use of a scanning electron microscope
- e. Electrophoresis
- f. Mass-spectrometric analysis

5.2 Summary of retrieved components

The 47 components used in the initial study were retrieved from 24 male and 23 female patients. All of the components had reached the end of their usable life, in-vivo, after an average of eight years and three months in service and were manufactured from UHMWPE. Of the 47 components 9 were polyethylene liners implanted with metal-backing and the rest (38) were implanted only with PMMA cement. The service life in these cases varied from 1 year to 23 years and 5 months in-vivo. The retrieved acetabular cups were from various manufacturers. In all cases, the reason for revision surgery was a loose acetabular cup causing severe pain and discomfort to the patient.

All the retrieved cups showed signs of excessive wear and/or creep. In four cases, there were catastrophic failures. For an example of these catastrophic failures, see Figure 5.1. In the second cup, something was wrong with the material (Figure 5.2), as the material started delaminating within a year after implantation. These details are discussed later.



Figure 5.1: Fractured cup



Figure 5.2: A cup with delamination

5.3 Visual inspection of retrieved acetabular cups

The cups were visually inspected to try to identify any obvious defects in the retrieved components. The findings of the visual inspection are as follows:

All the cups showed some form of wear and/or creep. It is difficult to distinguish exactly between wear and creep. It was therefore assumed that a combination of both occurred. The extent of wear/creep varies depending on the number of years in-vivo. The position of the maximum wear/creep correlated with data found in literature (Sychterz et al., 1996; Jasty et al., 1997; Schmalzried et al., 1999). Figure 5.3 shows a cross section of an acetabular cup showing the amount of wear/creep and Figure 5.5 provides a schematic presentation of the wear area.

Visible in all of the cups was some form of debris deposited outside the high load area (see Figures 5.4 and 5.5). The debris was attached to the base material and could not be removed. When viewed under a magnifying glass, this debris appeared like “molten” material deposited on a cold surface. Also visible under the magnifying glass were craters and pieces of material with an irregular pattern similar to the surface of an orange. (See paragraph 4.3.3.) At this stage of the investigation, it was felt that this debris holds the key to what is actually happening on the running surface and therefore warranted an in-depth investigation.



Figure 5.3: Cross section of cups showing an amount of wear/creep



Figure 5.4: Deposited debris – coloured with liquid dye penetrant to make it more visible

As mentioned in paragraph 5.2, four of the retrieved cups showed catastrophic failures. The first cup had fractured and it also showed some signs of cracking in other areas. It appears that the fatal crack started from a crater caused by material being extruded from the base material in the main adhesion wear area (See Figures 5.6 and 5.7).

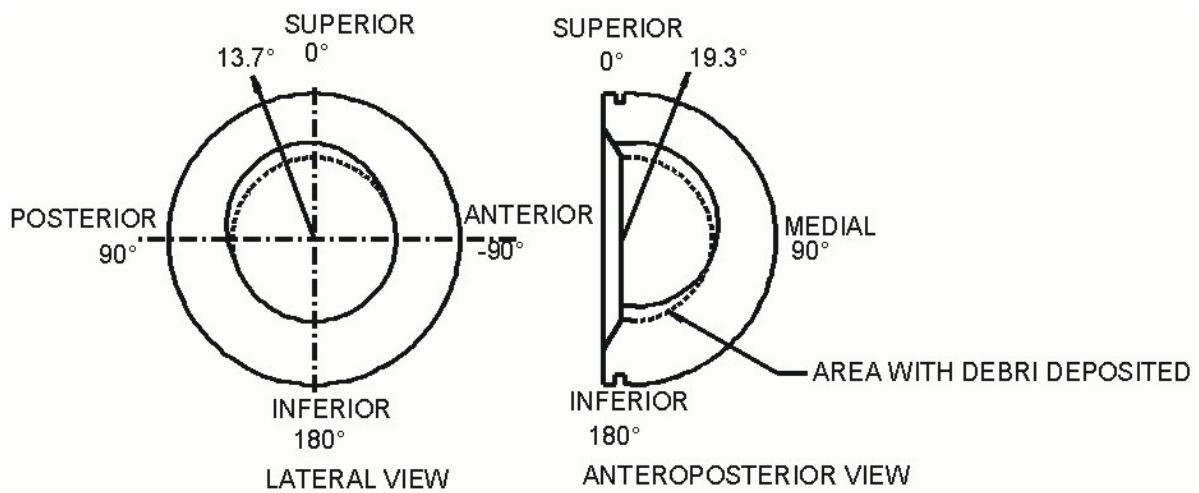


Figure 5.5: Wear pattern in retrieved cups with area of debris deposits indicated

The cups were treated with dye penetrant to make the defects more visible. The cup, shown in Figure 5.2, failed after only one year in-vivo. When inspected, using a magnifying glass, it appeared that the material was defective and that it had delaminated as can be seen in Figure 5.2. A possible explanation for this delamination is that during the manufacturing process a harder layer on the surface had formed, similar to case hardening in steel. This can be a result of incorrect cooling, incorrect cutting speed or a blunt cutting tool. When the acetabular cup is in service, contact stresses are generated at a distance below the surface (Boresi & Sidebottom, 1985). Repetitive loading may then result in fatigue failure at the depth below the surface where the contact stresses are maximised, causing the top layer of material to delaminate.

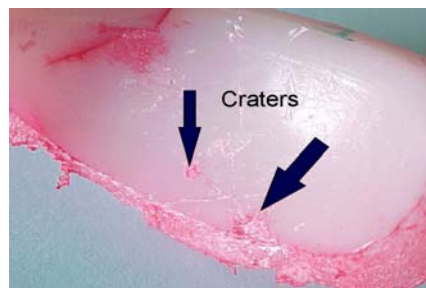


Figure 5.6: Crater in fractured cup

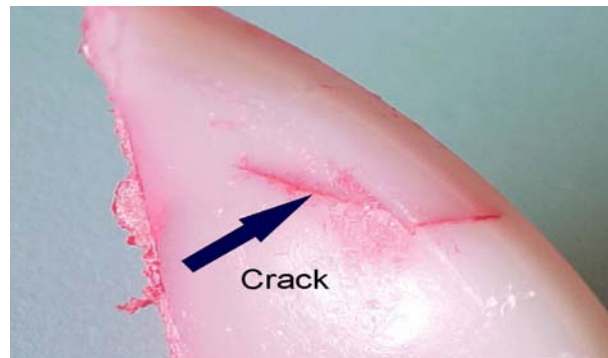


Figure 5.7: Cracking in fractured cup

Apart from the wear/creep and the catastrophic failures, a number of other types of failure were identified in the retrieved acetabular components. The first is interference between the acetabular cup and the neck of the femoral component as shown in Figure 5.8. This was caused by misalignment in-vivo. This misalignment may be a result of problems during surgery or, more often, the impingement damage is secondary to a cup loosened due to osteolysis with the resulting movement of the cup causing misalignment. The result is that the neck of the femoral component interferes with the acetabular cup removing pieces of material (UHMWPE and PMMA cement). This debris can find its way into the bearing area where it can cause third-body wear, as discussed earlier. The larger pieces of UHMWPE removed from the rim of the cup, can also cause further osteolysis.

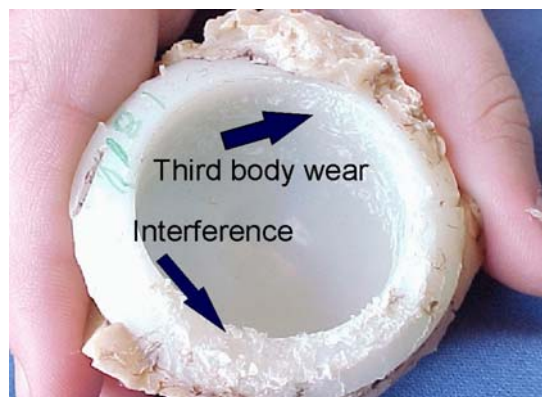


Figure 5.8: A cup with evidence of interference and subsequent damage

Foreign particles can find their way onto the bearing surface area. In one of the cups, a steel chip or shaving was trapped on the bearing surface and subsequently pressed into the softer polyethylene by the zirconium femoral head as shown in Figure 5.9. The origin of the steel particle is not known, but it can only be from the surgical tools because the femoral head was not manufactured from steel but zirconium and the femoral stem was manufactured from titanium. The piece of steel was embedded in the cup and did not cause the failure. Defects originating from surgical procedures will not be investigated further in this study.

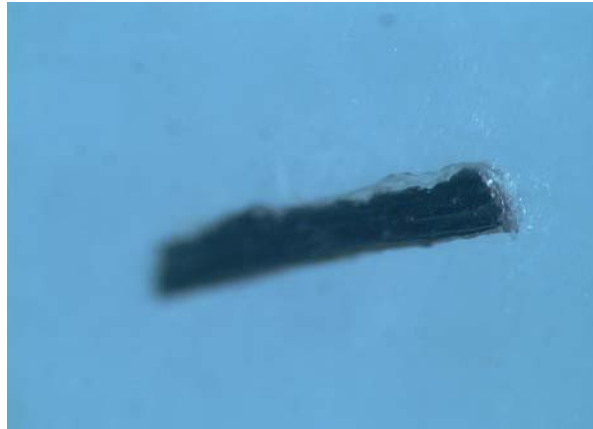


Figure 5.9: Piece of steel embedded in polyethylene (magnification x 40)

Another defect detected visually was an acetabular cup with severe plastic flow visible round the rim of the cup together with cracks running radially from the area of plastic flow. This is shown in Figures 5.10 and 5.11. The cause of this plastic flow is discussed later. In Figure 5.11, a vernier is held against the “flowed” material to indicate the extent of flow that had taken place.

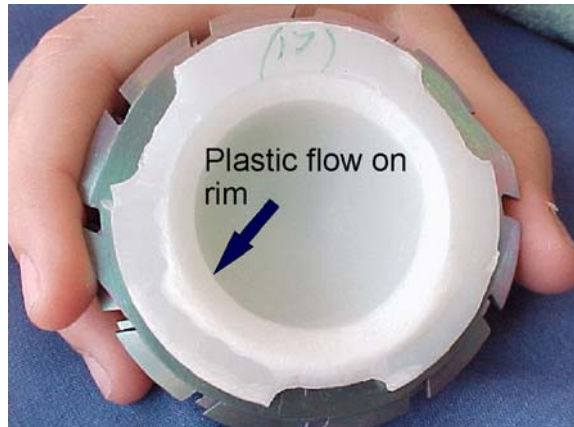


Figure 5.10: Plastic flow on the rim of cup

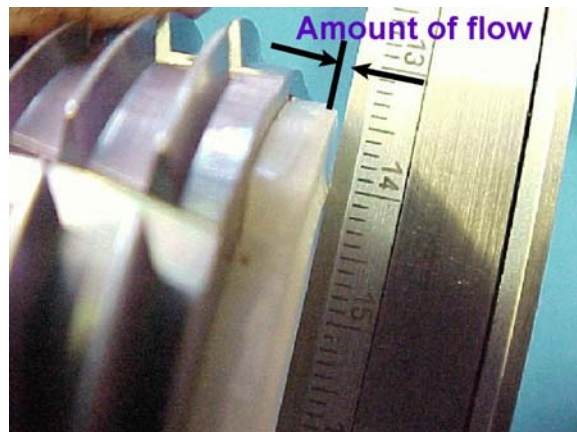


Figure 5.11: Amount of plastic flow

In virtually all of the cups, a whitish deposit was found inside the cup on the bearing area. Owing to the transparent nature of this deposit, it was very difficult to photograph. However, in one of the cups, the deposit was so thick that it was possible to collect and study it using an electron microscope. The deposit was found to be sodium chloride, which was probably deposited after washing the wound and the retrieved component in theatre during revision surgery. (The result of the electron microscope investigation is attached in Annexure B.) This deposit has no influence on the failure analysis of this investigation and will be ignored. Figure 5.12 shows the acetabular cup with the white deposit collected. Also visible in this cup is a slight brown discolouration towards the rim of the cup. The brown discolouration was also

investigated further using an electron microscope. In paragraph 5.6 it will be shown that this is not a discolouration but a deposit on the inside of the cups.



Figure 5.12: White deposits on inside of cup and brown discolouring

Based on the visual inspection, it is clear that different modes of in-vivo failure were identified in the 47 acetabular cups investigated. Although there were other causes of failure (for example misalignment during surgery and poor bone stock resulting in early loosening of the cup), the majority of these cups had failed mechanically due to excessive wear and/or creep. The wear debris found on the inside of these cups suggested that the material has been forcefully removed from the bearing area under pressure and was then deposited outside the bearing area.

5.4 Inspection making use of dye penetrant

The retrieved cups have many surface defects as described earlier. To be able to investigate the extent of the damage and debris deposits in the cup, it was decided to make use of a liquid dye penetrant to colour the defects. The penetrant used was Chemserve Systems Ardrex 996P. The procedure used was to clean the cups making use of Chemserve Systems Ardrex 9PR551 and then to spray the cup with the penetrant. The penetrant was allowed to penetrate the defects for 30 minutes. The excess was then wiped off and the cup was cleaned, using Ardrex 9PR551.

By making use of this technique, all the visual observations made in paragraph 5.2 were confirmed. The material extruded from the bearing contact surface as well as debris removed from the bearing surface during operation can be seen in Figure 5.13.

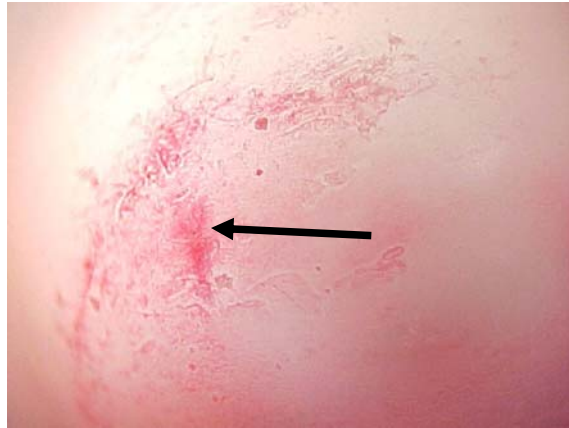


Figure 5.13: Deposited debris, resulting flow pattern and adhesion wear

The cup that had shown signs of plastic flow, Figures 5.10 and 5.11, was sectioned before treatment with the liquid dye penetrant. In Figure 5.14, the damage to the rim of the cup can be seen. With the dye penetrant test, it is clear that the material was subjected to conditions of plastic flow and had totally parted with the base material. What is interesting is that the flow had occurred over almost 80% of the rim circumference and not only in the high pressure area.

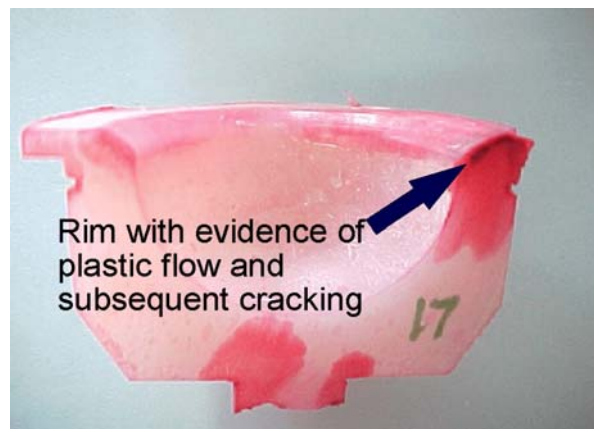


Figure 5.14: The rim of cup showing plastic flow on circumference of rim

Another feature of the plastic flow that became evident during the dye penetrant tests was that although the running surface looked polished, debris was deposited over the total bearing area. This deposited debris is visible in Figure 5.13. This led to the conclusion that the bearing surface is in actual fact not smooth but “pockmarked” due to debris deposited and then flattened and pressed into the bearing surface. The deposited wear debris is not evenly spread over the bearing surface as can be seen in Figures 5.13 and 5.15. Areas with higher concentrations of wear debris are found. These areas are investigated using a stereoscope, as discussed later.

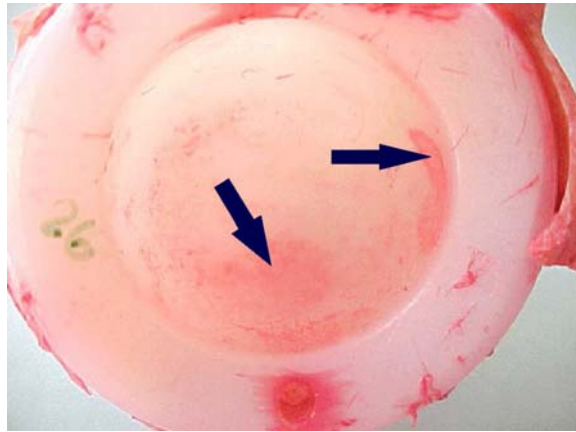


Figure 5.15: A cup with areas of higher concentration of debris

Another defect became clear when the acetabular cups were treated with the dye penetrant spray, namely areas of adhesion wear that had taken place. An acetabular cup with these areas clearly visible is shown in Figure 5.16 with a schematic layout of the positioning of this adhesion wear shown in Figure 5.17. This defect is also investigated further making use of the stereoscope as well as the electron microscope.

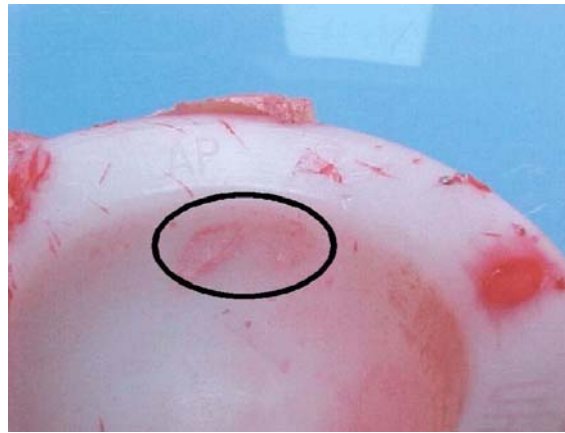


Figure 5.16: Close-up of the area of adhesion wear

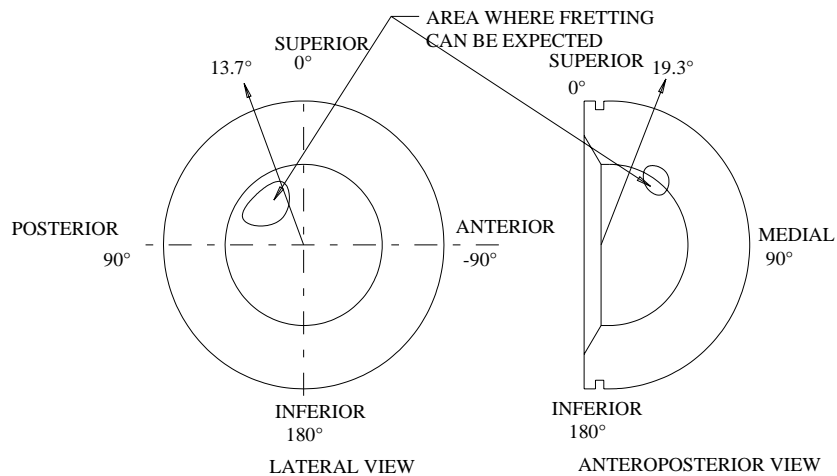


Figure 5.17: Schematic layout to indicate position of adhesion wear mark

At the position indicated in Figure 5.17, the contact stress of an implant is the highest. Adhesion wear is most likely to occur at this point where there is a combination of the different actions. The extension and flexion movement of the leg under load will force the lubricant from the bearing surface, while the abduction/adduction rotation and flexion movements of the leg will provide the energy input to cause adhesion wear or extrusion of material.

5.5 Investigation making use of stereoscope

Based on the investigations of paragraph 5.3 and 5.4, it became evident that more attention had to be paid to certain areas in the acetabular cups. The cups were examined using a Nikon stereoscope, equipped with a digital camera. It must be noted that in this investigation some parts of the photographs will be out of focus, because of the curvature of the cups.

The first cup investigated was the cup that had fractured (Figure 4.1). During the visual inspection and dye penetrant tests serious surface defects were visible as can be seen from Figure 5.6. When these defects were magnified, they turned out to be small craters formed when material was ripped from the base material. (See Figures 5.18 to 5.21.)

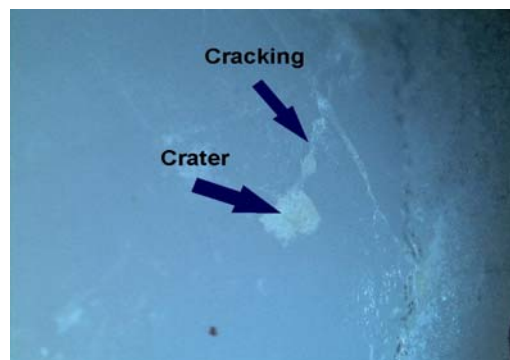


Figure 5.18: Magnification (x 10) of defect before treatment with dye penetrant

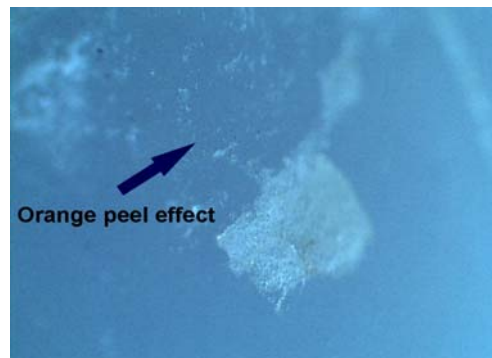


Figure 5.19: Magnification (x 20) of defect before treatment with dye penetrant

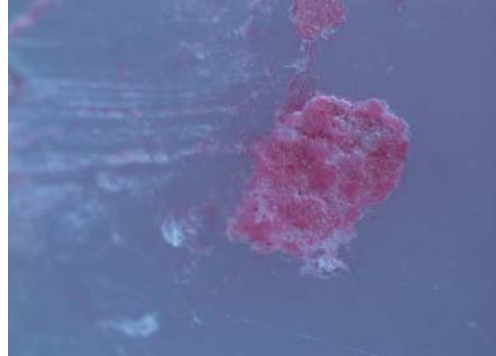


Figure 5.20: Magnification(x 20) of defect after treatment with dye penetrant

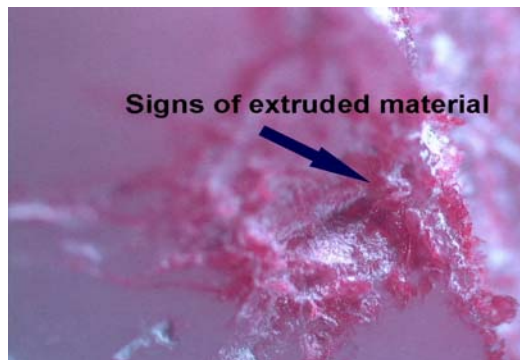


Figure 5.21: Magnification (x 40) of second crater after treatment with dye penetrant

The cracks seen in Figure 5.6 are now clearly visible in Figure 5.18. It is believed that the final fracture occurred after the cracks that had propagated from these defects (Figure 5.21) had extended beyond the critical crack length for this material. Also visible in Figure 5.19 are indications of the orange peel effect, as discussed earlier. Visible in Figure 5.21, is material that was extruded or forced out leaving the crater as indicated by the arrow.

If this material transfer takes place without evidence of high temperatures on the surface where the particles were removed or if the particles show no signs of having been extruded, the process will be referred to as adhesion wear. It is obvious that it will not always be possible to clearly distinguish between the two modes of failure, i.e. adhesion wear or extrusion. The starting point is, however, the same namely lack of lubrication and raised temperature (Hutchings 1992). Adhesion wear will occur when the movement of the femoral

ball in the acetabular cup is too small or too slow to cause sufficient heat build up to cause extrusion and/or plastic deformation.

The second failure that was investigated was the cup with material that flowed along the rim of the cup as shown in Figure 5.10. From the stereoscope photographs (Figures 5.22 and 5.23), it is clear that the material that flowed showed signs of separation from the base material. In this case the mass of the patient was approximately 70 kg. If it is assumed that the 32 mm diameter head made full contact, then the maximum pressure on the material is only approximately 1 MPa. This type of defect was found to be common in all of the specific type of acetabular cups retrieved.

The rest of the investigation concentrated on the wear debris found on the inside of the cups as well as the “pockmarked” bearing surface.

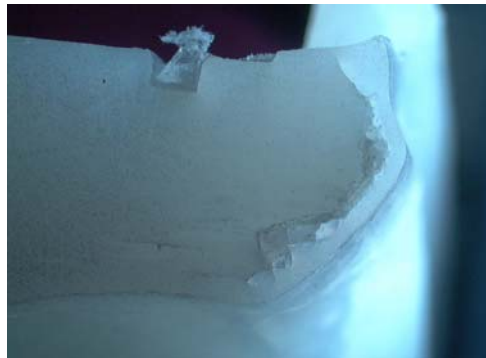


Figure 5.22: Magnification (x 10) of defect on rim of cup



Figure 5.23: Magnification (x 20) of defect on rim of cup

As described in paragraphs 5.2 and 5.3, wear debris is normally seen just outside the bearing surface. Photographs as shown in Figures 5.24 through 5.27 were taken to show what the debris typically looks like.



Figure 5.24: Magnification (x 10). From this photograph the white irregular shape of material deposited is clearly visible



Figure 5.25: Magnification (x 20). The arrow points to a droplet of material which was either extruded from the bearing surface or was flattened after being ripped out of the surface

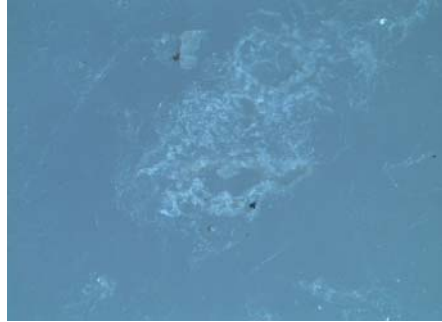


Figure 5.26: Magnification (x 20). The photograph shows softened/extruded debris with what looks like craters in between. Note the irregular shape of the deposit after being flattened by the ball.

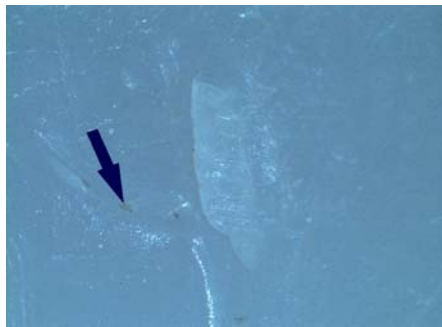


Figure 5.27a: Magnification (x 40): The results from Figure 5.25 are investigated further. It can be seen that the white piece of material appears not to have been totally removed from the base material, as there are no sharp edges on one side.

The indication is that material was extruded from the bearing surface. Some brown discolouring/deposit (indicated by arrow) can be seen in the grooves where material flowed over it. The schematic layout in Figure 5.27b shows a cross section of the described surface phenomenon.

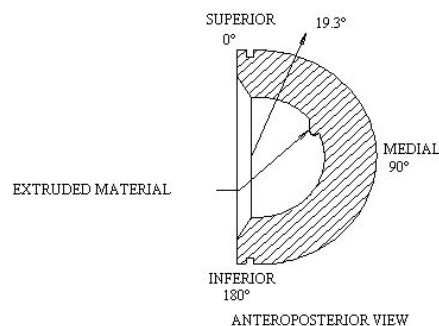


Figure 5.27b: Schematic of material extruded from the bearing surface

A further investigation was conducted into what looked like debris deposited on the bearing surface as shown in Figure 5.15. Under close examination with the stereoscope, it was found that the material was not deposited in these areas but that a skin or surface layer of the material was actually ripped off (see Figures 5.28 and 5.29). The machining marks are still partly visible. The extruded edges are also visible. Owing to the hardness and smoothness of the femoral head, these sheared off and flattened particles came loose, as was also found during the experimental tests. The debris showed up as a whitish deposit (see Figure 5.24).

Borne out by this study, is the resemblance between the wear pattern and the “butterfly” stress pattern as calculated and described by Wang et al. (1997) and Bennet et al. (1996), and shown in Figure 5.28.

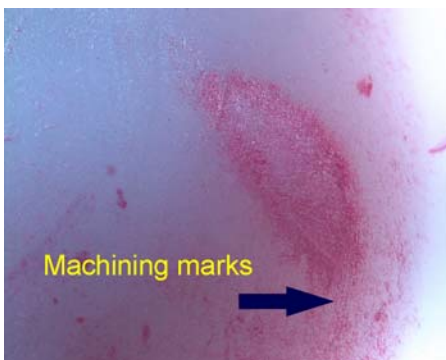


Figure 5.28: Magnification (x 20) of wear area on bearing surface



Figure 5.29: Magnification (x 40) of wear on bearing surface area

5.6 Electron microscope investigation

In some of the retrieved cups, a brown discolouration was visible, as noted in paragraph 5.2. What is very noticeable from the retrieved samples was that this discolouration again occurred just outside the boundary line of the bearing surface. The brownish discolouration was found attached to the polyethylene and could easily be scratched off. When investigated under a stereoscope no further detail could be detected.

The brown discolouring in the retrieved acetabular cups was then investigated under an electron microscope. This is shown in Figure 5.30. Under smaller magnification, the brown discolouring appears as tiles that are laid down on the polyethylene, while under higher magnification the structure of the brown discolouring is typically that of a protein, as shown in Figure 5.31.

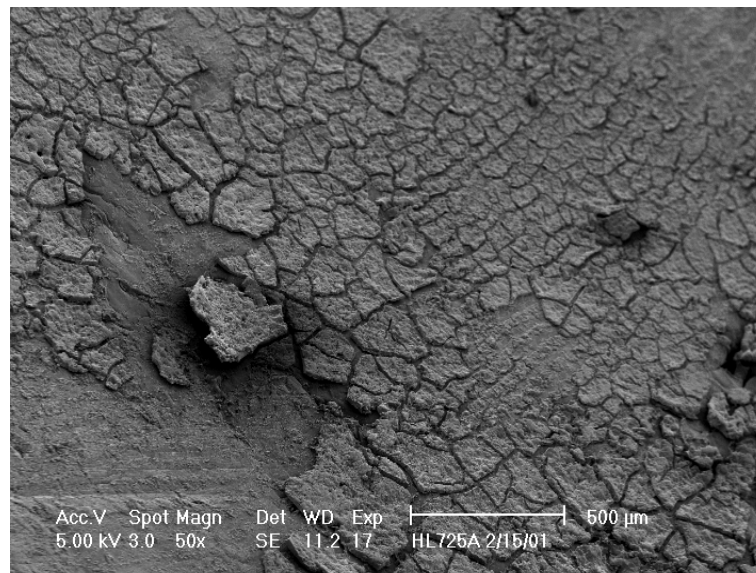


Figure 5.30: Electron microscope investigation of a brown layer on inside of cup (magnification x 50)

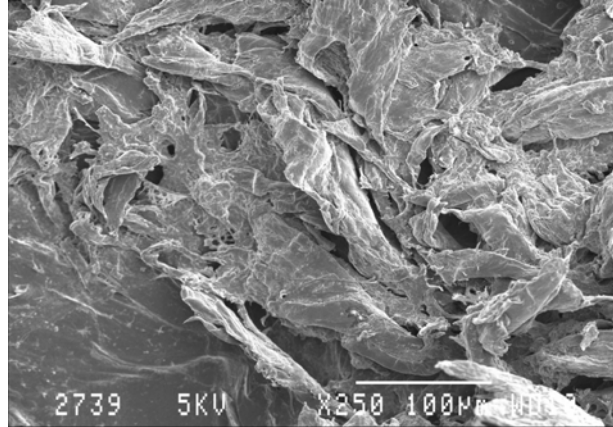


Figure 5.31: Electron microscope investigation of the brown layer on inside of the cup (magnification x 250)

As a first step in eliminating polyethylene as the observed texture, two samples were prepared of which one was deformed under compression, for investigation under the electron microscope (see Figures 5.32 and 5.33).

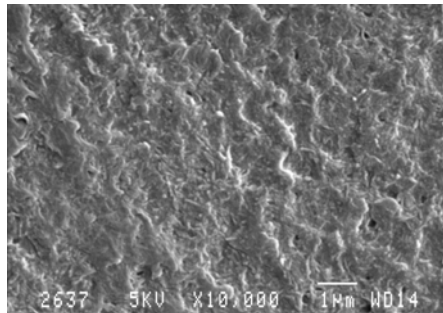


Figure 5.32: Electron microscope image of virgin UHMWPE sample (magnification x 10 000)

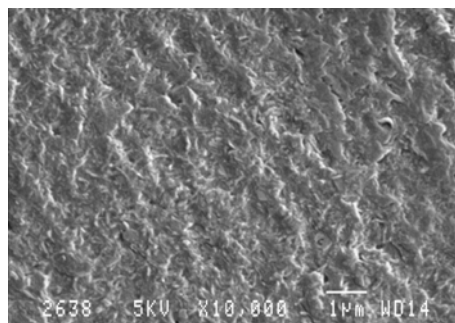


Figure 5.33: An electron microscope image of deformed UHMWPE (magnification x 10 000)

If the images in Figures 5.32 and 5.33 are compared, no difference can be seen between the virgin and deformed material, even under a magnification of 10000. The structure of the brown discolouring is totally different from that of the virgin or deformed UHMWPE and therefore the composition of the brown layer will have to be determined. (See paragraph 5.6.) The rest of the photographs for the electron microscope investigation into the brown discolouring can be seen in Annexure C and the photographs investigating the structure of UHMWPE can be seen in Annexure D.

To allow for smooth operation from the first day after implantation, the acetabular component is manufactured to allow for a maximum clearance of 0.5 mm on the diameter of the femoral ball. The allowance for the fit together with the angle at which the load is transferred into the joint results in an off-centre wear pattern as shown in Figure 5.34.

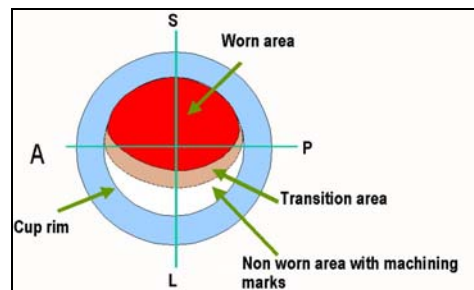


Figure 5.34: Anatomical cup orientation with position of worn area

The surface finish specified for manufacturing of the cups is $0.8\mu\text{m}$. The result is a gramophone finish in the cup. This can clearly be seen in Figure 5.35 and with a larger magnification in Figure 5.36. This finish is independent of whether the cup is crosslinked or not, as the crosslinking is done as the final step in the manufacturing.

These machining marks with the accompanying polyethylene debris attached to them are now subjected to a fatigue loading when the joint is in operation. This dynamic loading causes a varying shearing action on the polyethylene, resulting

in the separation of the particles. A typical particle separated from the base material can be seen in Figure 5.37.



Figure 5.35: Machining marks visible in acetabular cup

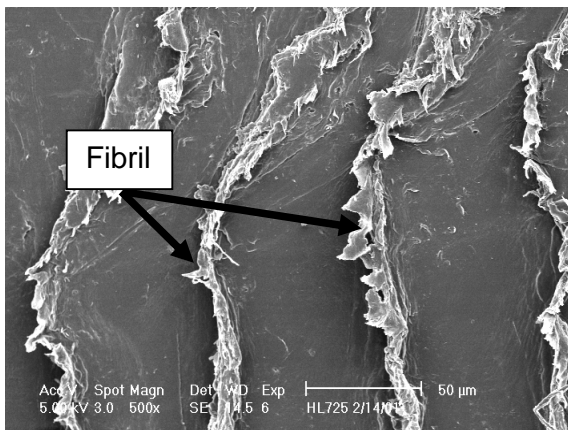


Figure 5.36: Machining marks on inside of cup (magnification x 500)

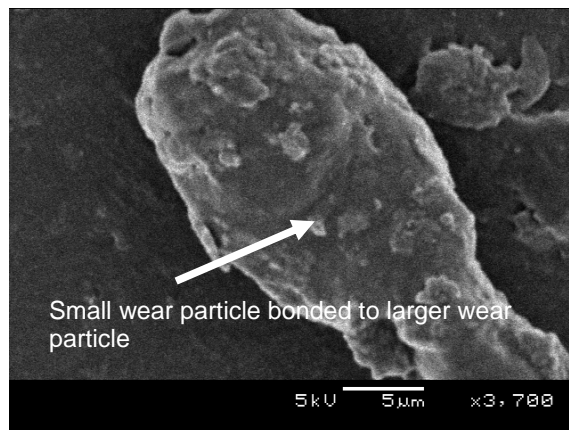


Figure 5.37: Wear particle entrapped in cup (magnification x 3 700)

These particles ripped from the base material, result in abrasion wear as can be seen in Figures 5.38 and 5.39.

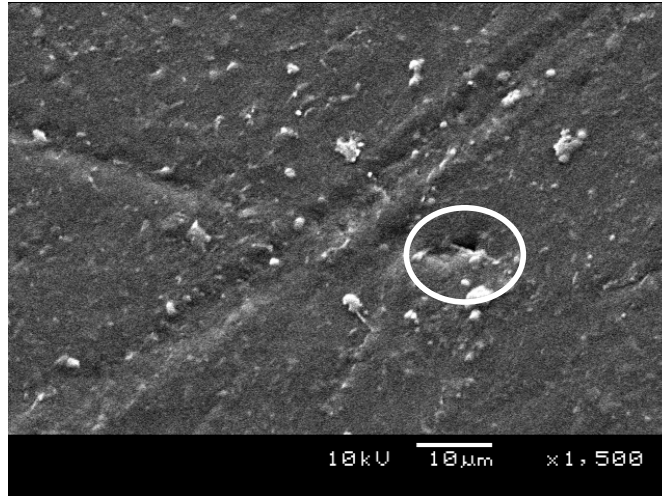


Figure 5.38: Surface of acetabular cup with scratches due to third-body wear (magnification x 1 500)

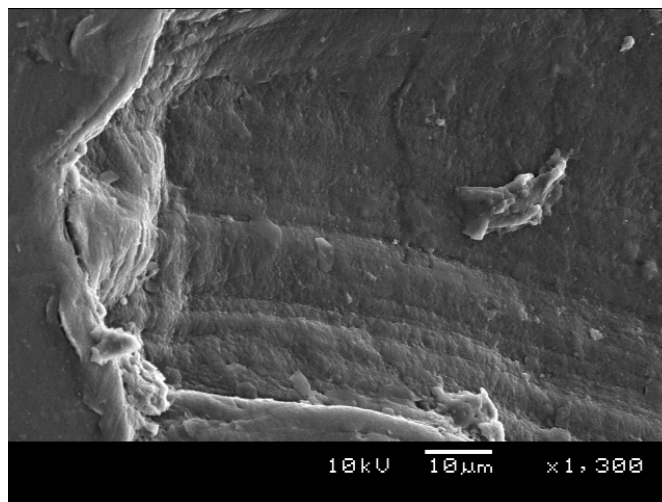


Figure 5.39: Abrasion wear on acetabular cup (magnification x 1 300)

To ensure that the scratches, as can be seen in Figure 5.38, were not a result of PMMA cement entering the bearing area, an electron microscope study with back scatter analysis was performed. In doing this analysis, the base material (UHMWPE) is compared with the particle lodged in the base material (encircled in Figure 5.38) at the end of the scratch to see if the particle is the same or if the

particle is from a different source. The result of this back scatter analysis can be seen in Figure 5.40.

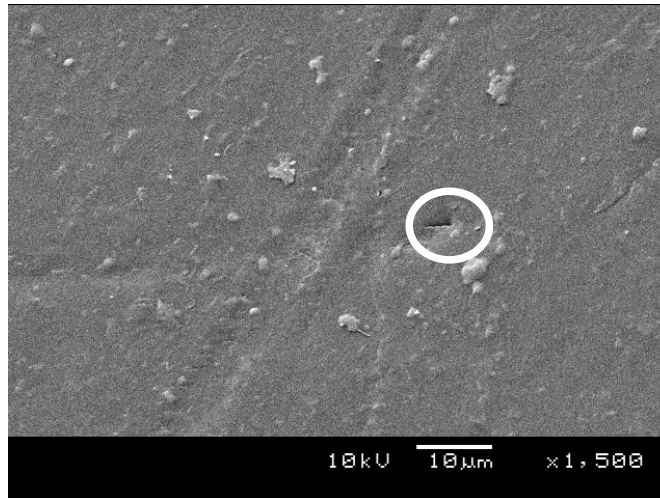


Figure 5.40: Back scatter analysis on wear particle lodged at end of abrasion wear in acetabular cup (magnification x 1 500)

From the back scatter analysis, as presented in Figure 5.40, in conjunction with a stereoscope investigation it can be concluded that the particle responsible for the abrasion wear as shown in Figures 5.38 and 5.39 was indeed an UHMWPE particle that was dislodged from the bearing area as foreign particles are now showing up in the abrasion area. In the area encircled in Figure 5.40, a cavity is also exposed penetrating into the acetabular cup. This type of cavity can create a stress raiser which can lead to early fracture of the acetabular cup.

A further analysis was done making use of the electron microscope. In Figure 5.41 an acetabular cup is shown with defects similar to the adhesion wear defects shown in Figures 5.28 and 5.29. The aim of this analysis was to gain a better understanding of the detail inside these adhesion wear areas.



Figure 5.41: Adhesion wear area in acetabular cup

The area as indicated in Figure 5.41 was cut out and gold-plated to be analysed in the electron microscope. The result of this analysis can be seen in Figures 5.42 and 5.43.

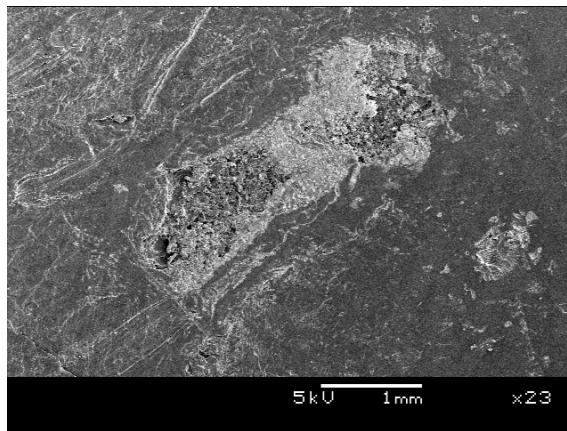


Figure 5.42: Electron microscope photograph of adhesion wear in acetabular cup (magnification x 23)

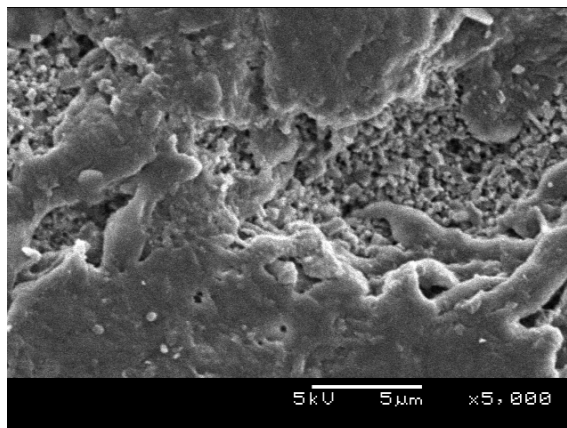


Figure 5.43: Adhesion wear area under higher magnification (magnification x 5 000)

From the data as presented in Figures 5.42 and 5.43, it is clear that the top layer of the material was ripped off by adhesion to the femoral head exposing the deeper part of the UHMWPE in the acetabular cup.

A second cup with a similar adhesion defect (visually) was also prepared for analysis in the electron microscope. The basic adhesion wear can be seen in Figure 5.44 with a higher magnification shown in Figure 5.45. The black markings on the picture are placed there to enable quicker detection of the defect under the electron microscope.

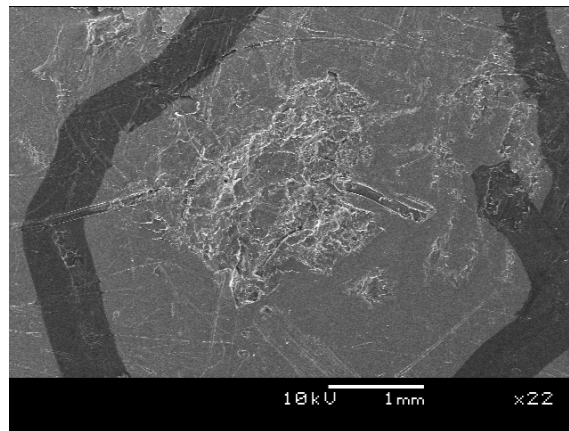


Figure 5.44: Adhesion wear defect under electron microscope (magnification x 22)

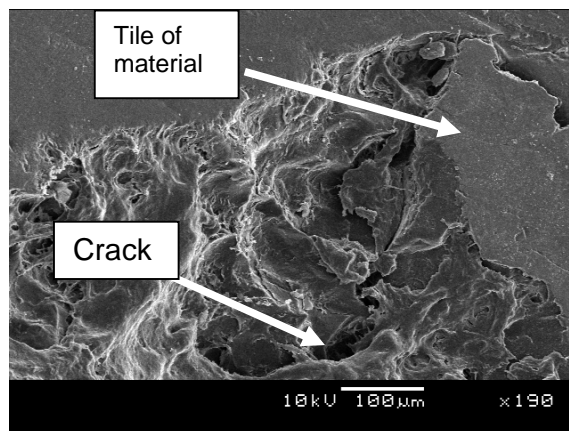


Figure 5.45: Adhesion wear under higher magnification (magnification x 190)

In the top right-hand corner of adhesion wear defect as presented in Figure 5.45 again it is clear that the top surface of the bearing area has been ripped off by adhering to the femoral head exposing the deeper part of the UHMWPE. It

would appear that the area was exposed by ripped-off tiles of UHMWPE. A tile at the verge of being dislodged is shown in Figure 5.45. Also visible in Figure 5.45 is the start of a deep crack propagating into the base of the acetabular cup. This crack will likewise create a stress raiser that may lead to early fracture of the acetabular cup.

During this investigation, areas where plastic flow of the base material had occurred were also exposed, as can be seen in Figures 5.46 and 5.47.

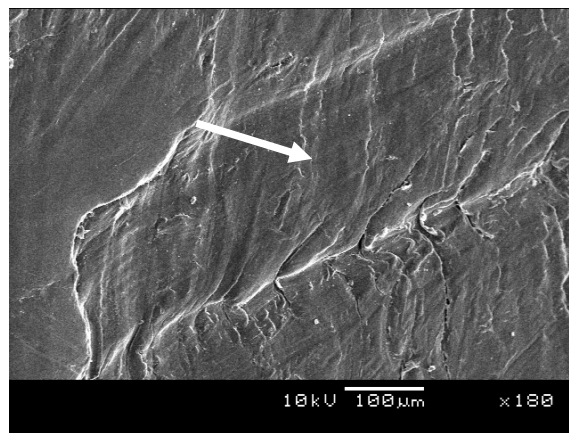


Figure 5.46: Acetabular cup with visible plastic flow on bearing area (magnification x 180)

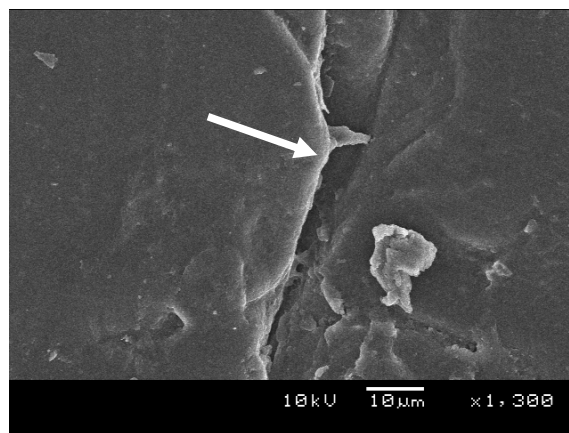


Figure 5.47: Plastic flow on bearing area under higher magnification (magnification x 1300)

The remainder of the pictures from the electron microscope analysis into the micro wear of the acetabular cup can be seen in Annexure E.

5.7 Electrophoresis

After reviewing the results of the electron microscope investigation it was decided to confirm the presence of proteins in the cup.

5.7.1 Method

Freshly retrieved cups were prepared by washing them with distilled water and then extracting the residue with an SDS-PAGE sample buffer, which is both a detergent and reducing agent for proteins. (<http://www.ehime-u.ac.jp/~manabet/act01eng.htm>; Rybicki & Purves, 1996). The samples were prepared within one hour after removal from the patients. During the preparation of the samples, it was already clear that there were proteins present as the SDS-PAGE buffer was cleaning off the brown discolouration. The samples dissolved with the SDS-PAGE buffer were then analysed by means of electrophoresis. (This work as well as the mass-spectrometric analysis was done by Mr Ben Mans of the Department of Biochemistry, University of Pretoria.)

5.7.2 Results

A sample of the results obtained for samples from the different acetabular cups, can be seen in Figure 5.48. Column 1 defines the markers to indicate the molar mass of the proteins. Column 2 is the analysis of a sample of synovial fluid retrieved from a patient. From the analysis of the fluid, it can be seen that there is a concentration of protein at 67 kDa as well as at 30 and 14 kDa. From literature (<http://www.ehime-u.ac.jp/~manabet/act01eng.htm>; Rybicki & Purves, 1996), it is found that the protein with a mass of 67 kDa is human serum albumen (HSA). Columns 3 to 6 give the results of the samples taken from a cup retrieved from a 72-year-old male patient eight years after implantation. This prosthesis was fitted with a 28 mm zirconium femoral head. Column 7 is a separator. Columns 8 to 12 represent the results obtained for a cup retrieved from a 42-year-old male

patient 4 years after implantation. This prosthesis was fitted with a 32 mm alumina head. From the results in Figure 5.48, it is clear that in both cases there is a presence of HSA, although the presence is not as marked as in the synovial fluid. A possible explanation for this is that the mechanism of lubrication in the cup is boundary lubrication and therefore the deposits detected were accumulated over a period of time.

From the relevant literature, it has been found that the denaturation temperature for HSA is between 333 and 343 K (60 - 70°C) (Sulkowska, 1997). The denaturation pressure for HSA at 25°C is 100 MPa. At a load of 4 000 N and with the femoral ball protruding 0.1 mm into the polyethylene the pressure on the contact surface is about 19.3 MPa, which is only 20% of the denaturation pressure. It is not clear what the relationship between temperature and pressure is when considering denaturation. This will have to be investigated in follow-up studies.

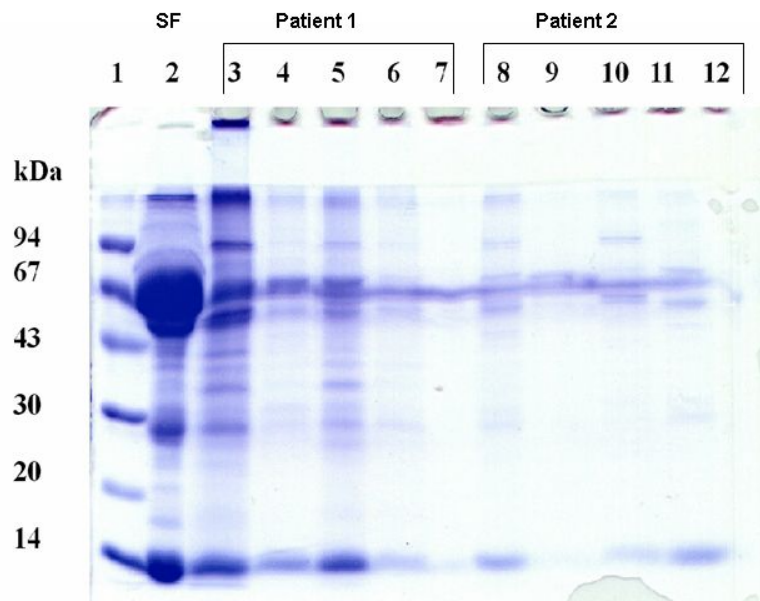


Figure 5.48: Electrophoresis analysis of synovial fluid and retrieved proteins from acetabular cups

The rest of the results can be found in Annexure F. The results indicate that proteins were deposited on the inside of the cups. Owing to deposited proteins, the coefficient of friction between the femoral head and the acetabular cup will probably increase, depending the percentage deposited proteins, resulting in more heat being released. Existing research has found that deposited proteins alone do not provide sufficient lubrication (Wang et al., 1998). These deposited proteins could possibly cause third body wear.

5.8 Mass-spectrometric analysis

Although it was not the aim of this study to determine the exact proteins deposited on the inside of the cups, it was still decided to do a mass-spectrometric analysis just to make sure that the brown discolouring that is visible is indeed protein-based.

5.8.1 Method

Mass-spectrometric analyses are done as follows:

1. A protein consists of a string of amino acids, coupled together in a chain with a specific sequence, for example:

NH₂-A-N-D-C-P-E-R-G-T-Y-V-K-L-M-N-E-G-V-C-D-D-Y-E-K-CO-OH

The left-hand side is called the N-terminal side while the right-hand side is called the C-terminal side (there is a free carboxyl acid group).

2. The above chain will have a specific mass which is determined as the sum of the masses of the different amino acids.
3. A specific enzyme is used to split proteins on a particular amino acid. The enzyme used during this test is tripsyn and it cleaves to the string on the C-terminal side of arginine (R) and lysine (K) that are both being positively charged amino acids.
4. If the protein, with an arrangement as in 1, is digested with tripsyn, fragments of each with their own unique mass will be formed, for example:

a. NH₂A-N-D-C-P-E-R-COOH Mr~660 Da

- b. $\text{NH}_2\text{-G-T-Y-V-K}$ Mr~440 Da
 - c. $\text{NH}_2\text{-L-M-N-E-G-V-C-T-D-D-Y-E-K-COOH}$ Mr~1430Da
5. Mass-spectrometric analysis only determines the masses of the generated peptides and with this example three peaks can be expected on positions 440, 660 and 1430.
 6. Because each protein has a unique amino acid arrangement, it will result in unique fragments. Because trypsin is also a protein, it can be digested into a specific fragment, and therefore a control experiment must be done.

5.8.2 Results

Five samples from the SDS-PAGE analysis were taken and put through the mass-spectrometric analysis. The result of one of the samples is shown in Figure 5.49. The rest of the results can be found in Annexure G. Two mass-spectrometric tests were done on each of the samples, explaining the first two lines on the graph. A control test was done for the trypsin, as explained above. The trypsin is represented by the third line (green) on the graph. In Figure 5.49, the trypsin line must be subtracted from the two lines above. The presence of amino acid fragments (after subtraction) forming proteins can be seen. What is also clear from the five different graphs is that the protein deposits vary from patient to patient.

Tissue retrieved during the same surgical procedure was dissolved using caustic soda. The solution was left for a minimum of four hours to allow segregation to take place. The light polyethylene wear particles will float on top of the solution. The top layer of the solution was decanted and passed through the 0.45µm filters to isolate the wear debris.

The biggest challenge during this investigation was to see the wear debris under the microscope, as the filters are white and the wear particles are transparent, therefore it was difficult to see the wear particles on the filter material. A decision was taken to spray the filter material with dye penetrant. The penetrant will penetrate the filter material, colouring it pink. The wear debris is not porous and thus the penetrant will hardly have an effect on the wear particles.

5.9.2 Results

The microscope revealed particles with extruded edges, namely particles that became sufficiently plastic to allow extrusion of fibre-like particles from the base material under the prevailing pressure. The retrieved particles are shown in Figures 5.51 to 5.54.

The formation of the wavy edges can be compared to defects found in the extrusion of aluminium. (See Figure 5.50.) The temperature of the thinner material drops at a faster rate than the temperature of the material in the middle. When the material in the middle shrinks during the cooling-off process, the thinner material on the outside that has already cooled off is put under compression causing wavy edges.

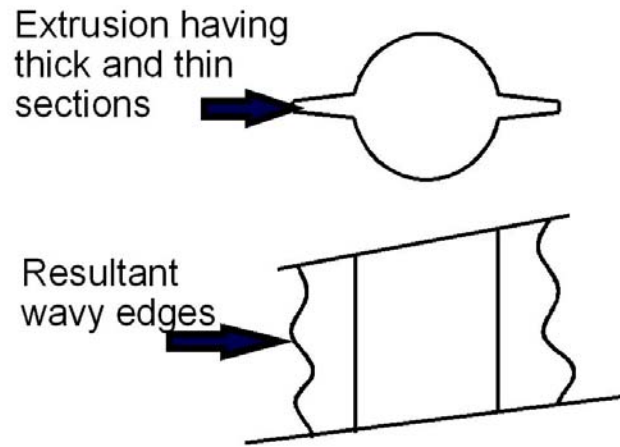


Figure 5.50: Schematic explanation of formation of jagged/wavy edges during extrusion

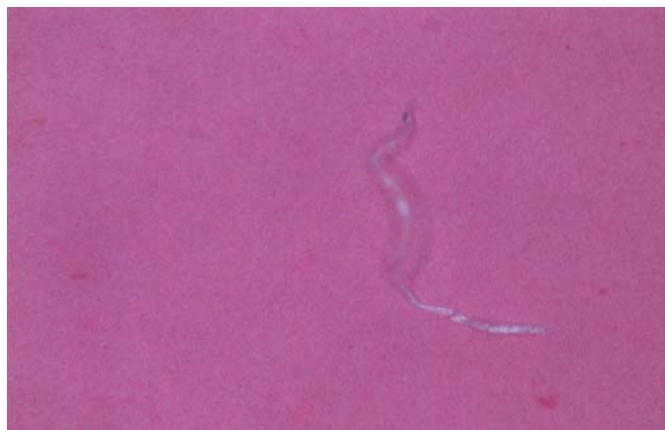


Figure 5.51: Particles retrieved from tissue (magnification x 40)

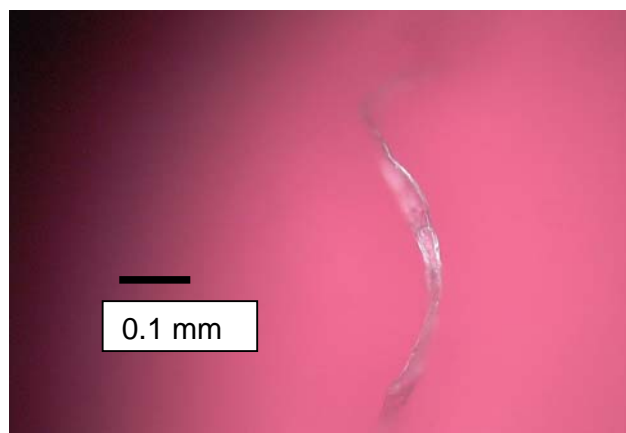


Figure 5.52: Retrieved particle (magnification x 100)

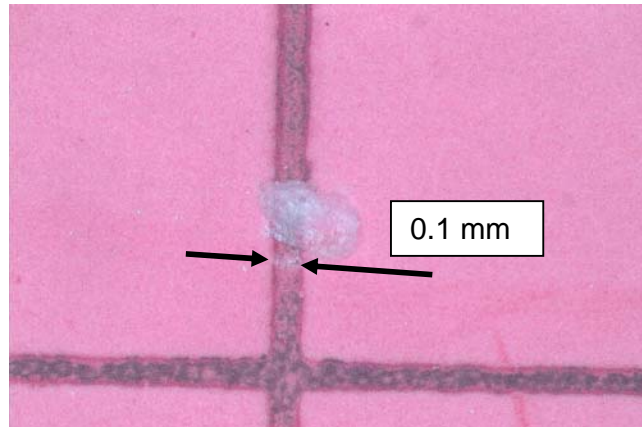


Figure 5.53: Retrieved particle (magnification x 40)

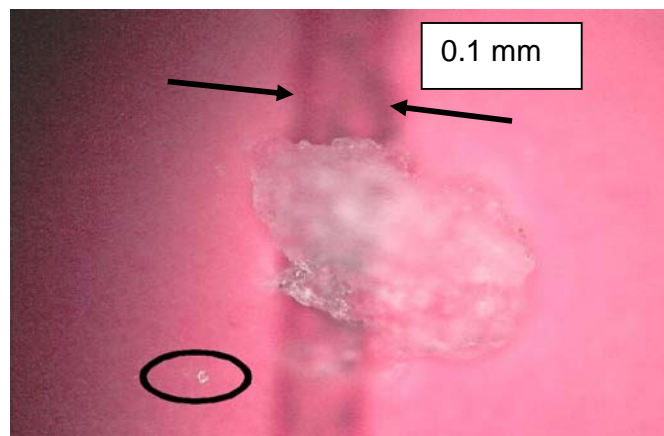


Figure 5.54: Retrieved particle (magnification x 100)

It should be noted that although particles smaller than $1\ \mu\text{m}$ are also visible, the particles shown in the above Figures are quite large. The large particle in Figure 5.54 is approximately 0.3 mm long. The small particles look like droplets rather than products of normal wear. One of these droplets can be seen (encircled) in Figure 5.54.

The extruded whisker shown in Figure 5.52 is almost 0.6 mm long. The full whisker is not shown in the picture as it curves up and down on the filter paper and is therefore difficult to get in focus.

In the work done by Schmalzried et al. (1997) and Maloney et al. (1995), different techniques were used to retrieve wear particles from tissue. The

emphasis in these studies is to quantify the amount and size distribution of wear debris released. This was done in an attempt to determine the amount of third-body wear present in the joints. Photographs were taken of the debris. Although the photographs were taken of debris smaller than $1\mu\text{m}$, the shape of the debris is exactly the same as found in the present study.