SEALING THE BONE-IMPLANT INTERFACE AROUND TOTAL HIP REPLACEMENTS USING GUIDED BONE REGENERATION

By

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Contents

Abstract

Acknowledgements

List of Figures

List of Tables

Chapter One

Chapter Two

Chapter Three

Chapter Four

Chapter Five

Chapter Six

Appendix I

References

Page number

III

IV

V

IX

1

39

48

67

102

175

181

191
The aim of this project was to prevent wear debris from reaching the interface of the acetabular cup and femoral component by the use of a partially occlusive e-PTFE membrane. This membrane would initially act as a physical seal, which would become incorporated by bone and soft tissue, forming a secondary biological seal. The hypothesis was that these physical and biological seals, would prevent the debris from accessing the interfacial tissues where they cause bone loss and implant loosening.

The biocompatibility of the membrane, and the glue used to attach the membrane was initially assessed by in vitro testing. Osteosarcoma cell proliferation over e-PTFE used with butyl-cyanoacrylate for 24 hours occurred at 20% the rate of controls. In a rabbit study, the membrane protected a femoral defect into which bone would grow more rapidly than an unprotected site. The second component of the rabbit study demonstrated that e-PTFE significantly (P<0.02) enhanced the osseo-integration of a trans-femoral titanium screw. A wear test characterised a surface roughness and morphology that wore at a known accelerated rate, producing particles of the same number, size and shape to those seen around human hip replacements after ten years.

An animal model was developed to test the hypothesis. The model would replicate the mechanisms of loosening where the effects of wear debris could be studied. Using femoral heads with the appropriate roughness, as determined by the wear test, a goat model produced the radiological and histological presentation of loosening as observed in human total hip replacements. Loosening was assessed by measurement of the radiolucent line, and attributable to wear debris by histological investigation. The e-PTFE membrane prevented acetabular implant loosening, to a statistical significance of 0.02 in a blinded assessment, when compared to the control groups. Loosening of the first 5mm of the proximomedial aspect of the femur was also prevented.

To summarise, we prevented wear particle induced osteolysis in the acetabular component of a loosening goat model by using an e-PTFE membrane to seal the bone-cement interface.
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The irony of ‘Acknowledgements’ is that they tend to give more of an indication of the person writing them, than the people being written about!
<table>
<thead>
<tr>
<th>Fig. No.</th>
<th>Caption</th>
<th>Page number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Schematic for Membrane Placement</td>
<td>16</td>
</tr>
<tr>
<td>1.2</td>
<td>Photoelectronmicrograph of e-PTFE surface. Low Power</td>
<td>29</td>
</tr>
<tr>
<td>1.3</td>
<td>Photoelectronmicrograph of e-PTFE surface at higher power</td>
<td>29</td>
</tr>
<tr>
<td>1.4</td>
<td>Photoelectronmicrograph of section through e-PTFE</td>
<td>30</td>
</tr>
<tr>
<td>2.1</td>
<td>Cell Proliferation in Elutant Dilution</td>
<td>44</td>
</tr>
<tr>
<td>2.2</td>
<td>Cell Proliferation over Different Surfaces</td>
<td>45</td>
</tr>
<tr>
<td>3.1</td>
<td>Schematic of Bone Defect</td>
<td>51</td>
</tr>
<tr>
<td>3.2</td>
<td>Schematic for the use of Guided Bone Regeneration in the Osseo-mechanical integration of prostheses</td>
<td>52</td>
</tr>
<tr>
<td>3.3</td>
<td>Control operative site</td>
<td>53</td>
</tr>
<tr>
<td>3.4</td>
<td>Test operative site covered with e-PTFE square</td>
<td>54</td>
</tr>
<tr>
<td>3.5</td>
<td>Woven Bone in Control site after 2 weeks</td>
<td>55</td>
</tr>
<tr>
<td>3.6</td>
<td>Woven Bone in Test site after 2 weeks</td>
<td>56</td>
</tr>
<tr>
<td>3.7</td>
<td>Lamellar Bone in Control site after 2 months</td>
<td>57</td>
</tr>
<tr>
<td>3.8</td>
<td>Lamellar Bone in Test site after 2 months</td>
<td>57</td>
</tr>
<tr>
<td>3.9</td>
<td>Osteoblasts in e-PTFE matrix</td>
<td>58</td>
</tr>
<tr>
<td>3.10</td>
<td>e-PTFE significantly enhanced Bone Regeneration</td>
<td>59</td>
</tr>
<tr>
<td>3.11</td>
<td>e-PTFE significantly enhanced osseointegration</td>
<td>60</td>
</tr>
<tr>
<td>3.12</td>
<td>Little bone regeneration into defect and around implant</td>
<td>62</td>
</tr>
<tr>
<td>3.13</td>
<td>Enhanced Bone Regeneration into Defect and up to Implant</td>
<td>62</td>
</tr>
<tr>
<td>3.14</td>
<td>Control Group with Fibrous Membrane around whole screw</td>
<td>63</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>3.15</td>
<td>Complete Bone integration of Implant in Test Group</td>
<td>63</td>
</tr>
<tr>
<td>3.16</td>
<td>Fibrous tissue in contact with Titanium in Control groups</td>
<td>64</td>
</tr>
<tr>
<td>3.17</td>
<td>Bone directly against Titanium Implant in Test Groups</td>
<td>64</td>
</tr>
<tr>
<td>4.1</td>
<td>Different Abrasive Wear Mechanisms</td>
<td>69</td>
</tr>
<tr>
<td>4.2</td>
<td>Profilometer Schematic</td>
<td>72</td>
</tr>
<tr>
<td>4.3</td>
<td>Average Roughness Trace</td>
<td>73</td>
</tr>
<tr>
<td>4.4</td>
<td>Test Rig for Abrasive Wear Test</td>
<td>80</td>
</tr>
<tr>
<td>4.5</td>
<td>Test Schematic</td>
<td>81</td>
</tr>
<tr>
<td>4.6</td>
<td>Rougher Papers Produce a Higher Average Roughness</td>
<td>84</td>
</tr>
<tr>
<td>4.7</td>
<td>Scratched CoCr Surface (X100)</td>
<td>84</td>
</tr>
<tr>
<td>4.8</td>
<td>Scratched CoCr Surface (X1500)</td>
<td>85</td>
</tr>
<tr>
<td>4.9</td>
<td>Shot-Blasted Surface with Entirely Different Morphology</td>
<td>85</td>
</tr>
<tr>
<td>4.10</td>
<td>Rougher surfaces increase the Height of the Asperities</td>
<td>86</td>
</tr>
<tr>
<td>4.11</td>
<td>Adjacent Peaks Further apart in Rough &amp; Polished surfaces</td>
<td>87</td>
</tr>
<tr>
<td>4.12</td>
<td>Volume Loss from Polyethylene Discs(1)</td>
<td>88</td>
</tr>
<tr>
<td>4.13</td>
<td>Volume Loss from Polyethylene Discs(2)</td>
<td>89</td>
</tr>
<tr>
<td>4.14</td>
<td>Weight Loss from Polyethylene Discs(1)</td>
<td>90</td>
</tr>
<tr>
<td>4.15</td>
<td>Weight Loss from Polyethylene Discs(2)</td>
<td>90</td>
</tr>
<tr>
<td>4.16</td>
<td>Wear Factor Expressed for Each Grit Paper</td>
<td>91</td>
</tr>
<tr>
<td>4.17</td>
<td>SEM Photomicrograph Showing Adherent Poly Particle</td>
<td>92</td>
</tr>
<tr>
<td>4.18</td>
<td>Graph Demonstrating Extent of Polishing of CoCr pins</td>
<td>92</td>
</tr>
<tr>
<td>4.19</td>
<td>Polyethylene Particles from Grit Paper 240</td>
<td>93</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>4.20</td>
<td>Polyethylene Particles from Grit Paper 600</td>
<td>94</td>
</tr>
<tr>
<td>4.21</td>
<td>Polyethylene Particles from Grit Paper 1200</td>
<td>94</td>
</tr>
<tr>
<td>4.22</td>
<td>Polyethylene Particles at a Larger Mag. from Grit Paper 600</td>
<td>95</td>
</tr>
<tr>
<td>4.23</td>
<td>Polyethylene Particles at a Larger Mag. from Grit Paper 240</td>
<td>95</td>
</tr>
<tr>
<td>4.24</td>
<td>Graph of Polyethylene Particle Distribution</td>
<td>97</td>
</tr>
<tr>
<td>5.1</td>
<td>Dimensions of e-PTFE membranes used for implantation</td>
<td>105</td>
</tr>
<tr>
<td>5.2</td>
<td>Intra-Operative View of Control Hip Insertion</td>
<td>107</td>
</tr>
<tr>
<td>5.3</td>
<td>Intra-Operative View of Test Hip Insertion</td>
<td>108</td>
</tr>
<tr>
<td>5.4</td>
<td>Sectioning Criteria of Goat Hip</td>
<td>114</td>
</tr>
<tr>
<td>5.5</td>
<td>Scanned Acetabular section radiograph</td>
<td>117</td>
</tr>
<tr>
<td>5.6</td>
<td>Average Roughness of Femoral Heads</td>
<td>122</td>
</tr>
<tr>
<td>5.7</td>
<td>Mean-Peak-to-Valley Distances of Femoral Heads</td>
<td>123</td>
</tr>
<tr>
<td>5.8</td>
<td>Mean Spacing Between Peaks of Femoral Heads</td>
<td>123</td>
</tr>
<tr>
<td>5.9</td>
<td>Wear of Rough heads in e-PTFE and Control Groups</td>
<td>125</td>
</tr>
<tr>
<td>5.10</td>
<td>Wear of Rough and Smooth heads</td>
<td>125</td>
</tr>
<tr>
<td>5.11</td>
<td>Polyethylene Particles in Hip Capsule</td>
<td>126</td>
</tr>
<tr>
<td>5.12</td>
<td>Numbers of Polyethylene Particles from rough heads only</td>
<td>127</td>
</tr>
<tr>
<td>5.13</td>
<td>Numbers of Polyethylene Particles</td>
<td>128</td>
</tr>
<tr>
<td>5.14a</td>
<td>Control 4, 8 &amp; 12 Month Post-Operative Radiographs</td>
<td>129-131</td>
</tr>
<tr>
<td>b &amp; c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.15a</td>
<td>Test 4, 8 &amp; 12 Month Post-Operative Radiographs</td>
<td>131-132</td>
</tr>
<tr>
<td>b &amp; c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.16</td>
<td>Sectional Radiograph of Hip Joint in Control Group</td>
<td>133</td>
</tr>
<tr>
<td>5.17</td>
<td>Sectional Radiograph of Hip Joint in Test Group</td>
<td>134</td>
</tr>
<tr>
<td>5.18</td>
<td>Loose Control Socket</td>
<td>135</td>
</tr>
<tr>
<td>5.19</td>
<td>Well Bonded Test Socket</td>
<td>135</td>
</tr>
<tr>
<td>Figure No.</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>5.20</td>
<td>Varying Extents of Loosening in Control and Test Groups</td>
<td>136</td>
</tr>
<tr>
<td>5.21</td>
<td>e-PTFE reduced the Extent of Loosening</td>
<td>137</td>
</tr>
<tr>
<td>5.22</td>
<td>Similar Extents of Loosening in Control and Test Groups, except proximo-medially</td>
<td>138</td>
</tr>
<tr>
<td>5.23</td>
<td>No Difference between Groups in Distal Femora</td>
<td>141</td>
</tr>
<tr>
<td>5.24</td>
<td>Control Cup with Roughened Head</td>
<td>142</td>
</tr>
<tr>
<td>5.25</td>
<td>e-PTFE sealed acetabular interface</td>
<td>143</td>
</tr>
<tr>
<td>5.26</td>
<td>Cement-Bone Interface at margin for e-PTFE group (X100)</td>
<td>144</td>
</tr>
<tr>
<td>5.27</td>
<td>Fibrous Tissue Interposing the Bone and Cement at the Cup Margin for the Control Groups</td>
<td>145</td>
</tr>
<tr>
<td>5.28</td>
<td>Polarised Image of Fibrous Tissue Interposing the Bone and Cement at the Cup Margin for the Control Groups</td>
<td>145</td>
</tr>
<tr>
<td>5.29</td>
<td>Osteolytic Wedge in control groups (X40)</td>
<td>146</td>
</tr>
<tr>
<td>5.30</td>
<td>Osteolytic detail from resorption wedge in control group (X200)</td>
<td>147</td>
</tr>
<tr>
<td>5.31</td>
<td>Macrophage detail from osteolytic wedge in control group (X200)</td>
<td>147</td>
</tr>
<tr>
<td>5.32</td>
<td>Macrophage detail showing intracellular birefringence (X200)</td>
<td>148</td>
</tr>
<tr>
<td>5.33</td>
<td>SEM micrograph of intimate lamellar bone-e-PTFE contact</td>
<td>149</td>
</tr>
<tr>
<td>5.34</td>
<td>e-PTFE bone interface, with osteoblastic penetration</td>
<td>150</td>
</tr>
<tr>
<td>5.35</td>
<td>Two cell populations growing either side of the e-PTFE membrane</td>
<td>144</td>
</tr>
<tr>
<td>5.36</td>
<td>Soft-tissue integrating e-PTFE interface (X100)</td>
<td>152</td>
</tr>
<tr>
<td>5.37</td>
<td>Resorption wedge of femoral component in control groups (X40)</td>
<td>153</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>5.38</td>
<td>Resorption wedge of femoral component in control groups (Polarised) (X40)</td>
<td>153</td>
</tr>
<tr>
<td>5.39</td>
<td>Swollen Macrophages at the Proximal End of the Medial Femoral Component in the Control Group (X200)</td>
<td>154</td>
</tr>
<tr>
<td>5.40</td>
<td>Macrophages containing Birefringent Material at the Proximal End of the Femoral Component in the Control Group (Polarised) (X200)</td>
<td>154</td>
</tr>
<tr>
<td>5.41</td>
<td>Fibrous tissue between the cement and bone in the Proximal Femur as noted in both test and control groups (X200)</td>
<td>155</td>
</tr>
<tr>
<td>5.42</td>
<td>e-PTFE and titanium in close approximation on the proximal femoral component, at the shoulder</td>
<td>156</td>
</tr>
<tr>
<td>5.43</td>
<td>Well-bonded cement mantle in control group</td>
<td>157</td>
</tr>
<tr>
<td>5.44</td>
<td>Collagen Layer in between Cement and Bone in Transverse Femur</td>
<td>158</td>
</tr>
<tr>
<td>5.45</td>
<td>Schematic of Intra-capsular structures</td>
<td>159</td>
</tr>
<tr>
<td>5.46</td>
<td>Swollen Macrophages and collagen (X100)</td>
<td>160</td>
</tr>
<tr>
<td>5.47</td>
<td>Intracellular Birefringence with collagen birefringence (X100)</td>
<td>160</td>
</tr>
<tr>
<td>5.48</td>
<td>Macrophages in hip capsule tissue (X400)</td>
<td>161</td>
</tr>
<tr>
<td>5.49</td>
<td>Birefringence within Macrophages (X400)</td>
<td>161</td>
</tr>
<tr>
<td>5.50</td>
<td>Scanning Electron Micrograph of a Plasma etched Resin section</td>
<td>162</td>
</tr>
<tr>
<td>5.51</td>
<td>Macrophages containing Birefringent material, surrounded by lymphocytes</td>
<td>163</td>
</tr>
<tr>
<td>Table No.</td>
<td>Caption</td>
<td>Page Number</td>
</tr>
<tr>
<td>----------</td>
<td>------------------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>1.1</td>
<td>Possible Complications Following THA</td>
<td>6</td>
</tr>
<tr>
<td>1.2</td>
<td>Cellular Distribution of Particulate Debris</td>
<td>13</td>
</tr>
<tr>
<td>1.3</td>
<td>Options for Replacement of Bone Defects</td>
<td>22</td>
</tr>
<tr>
<td>4.1</td>
<td>Average Roughness Values for Pins in First Test</td>
<td>86</td>
</tr>
<tr>
<td>4.2</td>
<td>Average Roughness Values for Pins in Second Test</td>
<td>88</td>
</tr>
<tr>
<td>5.1</td>
<td>Surgical summary of Goat Operative Procedures</td>
<td>110</td>
</tr>
<tr>
<td>5.2</td>
<td>Post-Operative Exercise Regime</td>
<td>110</td>
</tr>
<tr>
<td>5.3</td>
<td>Processing of <em>en bloc</em> Goat Sections</td>
<td>113</td>
</tr>
<tr>
<td>5.4</td>
<td>Results Format</td>
<td>121</td>
</tr>
<tr>
<td>5.5</td>
<td>Acetabular Analyses of Smooth and Rough Heads</td>
<td>137</td>
</tr>
<tr>
<td>5.6</td>
<td>Femoral Analyses of Smooth and Rough Heads</td>
<td>139</td>
</tr>
<tr>
<td>A1.1</td>
<td>Instruments used for primary total hip replacement</td>
<td>184</td>
</tr>
</tbody>
</table>
Chapter 1  Introduction to Total Hip Arthroplasty and Guided Tissue Regeneration

1.1 The Problem
1.2 The Evolution of Total Hip Arthroplasty
1.3 Total Hip Arthroplasty. Current Practices
1.4 Indications and Contraindications for Total Hip Arthroplasty
1.5 Complications following Total Hip Arthroplasty
1.6 Aseptic Loosening and Particulate Debris
1.7 Femoral Implant Failure Mechanisms
1.8 Acetabular Implant Failure Mechanisms
1.9 The Cellular Responses to Particulate Debris
1.10 The Role of Micromotion
1.11 The Hypotheses
1.12 The Concept of Guided Tissue Regeneration
1.13 Guided Bone Regeneration. A Historical Perspective
1.14 The Biological Basis for Guided Bone Regeneration
1.15 Expanded Polytetrafluoroethylene (e-PTFE)
1.16 Practical Aspects of Guided Bone Regeneration
1.17 The Use of Butyl-cyanoacrylate Adhesive
1.18 The Goal
1.1 THE PROBLEM

Osteoarthritis (OA) is a degenerative disorder of articular cartilage. When conservative treatment regimes have failed the damaged cartilage can be replaced by metallic and polymeric components, used for total hip arthroplasty (THA).

Although this is a very successful treatment, the problem is that these components do not remain attached to the bone in the long term, due to bone loss around the components.

Total hip arthroplasty (THA) is mainly a biomechanical treatment for painful hip conditions. Surgery aims to reconstruct the joint so that the centre of rotation of the prosthetic hip is in the same proximal-distal, and medial-lateral position as that of the original undiseased hip.
1.2 THE EVOLUTION OF TOTAL HIP ARTHROPLASTY

McKee and Charnley independently introduced the modern form of THA, although it was Charnley's "low friction" metal on polyethylene combination that proved more popular. This procedure is one of the landmark surgical successes of this century and since 1961 has remained the gold standard by which other types of implants are judged. Over the past forty years, no major improvement in the longevity of primary hip replacement has been made.

The problem of the hip osteoarthritis is not new in the bipedal human. Half a million years ago, the ‘Java’ man was afflicted, as well as the Ancient Egyptians and Romans (Jayson 1971). White performed an ‘excision arthroplasty' in 1822 at the Westminster Hospital of London, on a nine-year old boy with a septic and deformed hip. A deformed and ankylosed hip was treated in 1826, using an intertrochanteric osteotomy (Barton 1827). These techniques did not allow mobility in the long term. In order to maintain the range of motion obtained intraoperatively, interpositional arthroplasty was attempted, using muscles (Ollier 1885), gold foil (Jones 1921), and wooden blocks (Carnochan 1860). These did not last, since the materials wore off the bone surfaces.

An alternative design philosophy was adopted using a more radical reconstructive arthroplasty. These more extensive criteria involved removal of some, if not all, of the femoral head and neck. Materials for constructing hips included ivory (Gluck 1891), rubber (Deibert 1919), and methyl methacrylate (Jude et al. 1950). These prostheses broke down from the lack of structural integrity of the materials selected.
Wiles (1958), McKee (1951), Moore (1952), and Thompson (1952) introduced the metallic generation of prostheses. In 1960, Charnley introduced the concept of low-friction arthroplasty of the hip. This consisted of a stainless steel femoral head, and a polytetrafluoroethylene (PTFE) cup. The PTFE wore unacceptably, producing particulate PTFE debris that caused bone osteolysis. The PTFE was replaced by High Density Polyethylene. Wear of the acetabular component is now the longer term problem facing the survivorship of the components used in total hip arthroplasty.

1.3 TOTAL HIP ARTHROPLASTY. CURRENT PRACTICE

The current procedure involves removal of the femoral head and most of the neck, as well as enlargement of the acetabulum, without removal of the trochanter. The acetabulum is lined with a hemispherical UHMWPE (Ultra-High Molecular Weight Polyethylene) component.

The UHMWPE cup can be cemented directly into the acetabulum. Alternatively the component is metal-backed and locked into the acetabulum, with or without screws or cement. There are various types of finishes used on the metallic surface, including porous beads, wire mesh, or hydroxyapatite. Bipolar prostheses with the head captive within a metal “cup” allow free movement of the metal-backed cup over the acetabular surface.

The head of the femoral component tends to be cobalt chrome or ceramic, attached to a titanium, stainless steel, or cobalt chrome stem. The attachment is usually modular but can be heat shrunk. Diamond Like Carbon (DLC), is a very hard, scratch-resistant layer that may have a significant role in wear prevention. Currently there is research into the development of wear resistant polyethylene.
The femoral stem is inserted with or without cement depending on the design. Cemented stems can be produced with a layer of polymethylmethacrylate over the stem to aid cement attachment just after insertion. Uncemented stems are pre-coated with hydroxyapatite to promote early mechanical osseointegration. There are also custom designs that fit the medulla of the individual femur of each patient, produced by Computer Aided Design-Computer Aided Manufacture (CAD-CAM) methods.

1.4 INDICATIONS AND CONTRAINDICATIONS FOR TOTAL HIP ARTHROPLASTY

The introduction of THA was thought to be an alternative to procedures such as arthrodesis or excision arthroplasty. Patient selection is a major determinant for a successful outcome from THA. THA is indicated in many patients with arthritic diseases in which conservative medical management has failed.

Osteoarthritis is the most common indication for THA (Charnley and Cupic 1973, Harris and Sledge 1990). Other indications include rheumatoid or juvenile chronic arthritis, osteoarthritis secondary to, osteonecrosis, trauma, haemophilia or Gaucher's disease.

Contraindications for THA can be divided into those that precipitate septic loosening and those that do not. Any pre-operative acute or chronic systemic or local infection, diabetes mellitus, recent dental procedures, or localised skin lesions may all precipitate early septic loosening. Other potential risks include any nerve palsies to the lower limb, grossly obese patients, muscle wasting, extremes of age, compromised lower limb blood supply and extremes of fixed deformity.
Younger and middle-aged patients should be evaluated very carefully before an operation is considered. Successful THA remains largely an operation for the elderly, and until new technologies produce results to significantly prolong the life of Total Joint Prostheses, the responsible surgeon must bear this in mind.

1.5 COMPLICATIONS FOLLOWING TOTAL HIP ARTHROPLASTY.

A number of complications can occur post-operatively, and are divided into local and systemic, as well as immediate, early and late.

<table>
<thead>
<tr>
<th>Immediate</th>
<th>Early</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurovascular injuries</td>
<td>Dislocation</td>
<td>Aseptic loosening</td>
</tr>
<tr>
<td>Leg length discrepancy</td>
<td>Delayed infection</td>
<td>Heterotopic ossification</td>
</tr>
<tr>
<td>Acute infection</td>
<td></td>
<td>Femoral fractures</td>
</tr>
<tr>
<td>Systemic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotension from methyl-methacrylate monomer.</td>
<td>Deep-ven Thrombosis</td>
<td>Late haematogenous Infections</td>
</tr>
<tr>
<td></td>
<td>Fat Emboli</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1. Possible Complications Following THA

Hip pain is clinically associated with a failed implant. This can often be seen radiographically by radiolucent zones, calcar resorption, lytic lesions, cysts and masses; pseudotumours around the implant, indicating a 'radiographic failure.' At present the most common cause of joint failure is aseptic loosening (Amstutz et al. 1982, Beckenbaugh and Ilstrup 1978, DeLee and Charnley 1976, Freeman et al. 1982, Harris et al. 1982, Hunter et al. 1979, Kavanaugh et al. 1985, Stauffer 1982, and Sutherland et al. 1982).
Revision surgery is associated with more adverse complications than primary cases. Revision operations present with significant bone loss, requiring specialised prostheses and/or bone grafting.

There is no doubt that THA is a successful operation, making it very popular among both orthopaedic and general surgeons. The longevity of cemented hips in patients of 60 years and older is 90%, surviving at least ten years after surgery (Charnley and Cupic 1973). However, the upper limit to the working life of most prostheses is 15 years. The more the operation is performed, the more significant the failure rate becomes therefore increasing the need for a successful primary operation.

1.6 ASEPTIC LOOSENING AND PARTICULATE DEBRIS

Charnley's use of polytetrafluoroethylene cups produced a failure rate of over 95% (Charnley 1963). Having changed to UHMWPE, the next main problem was deep sepsis. Once the incidence of infection was reduced, endosteal lysis appeared and joints continued to loosen. McKee also noticed endosteal erosion, but considered the process to be mechanical in origin (McKee and Watson-Farrar 1966).

Harris et al. (1976) reported four cases of extensive localised bone lysis associated with the loosening of cemented total hip replacements in the absence of infection. This was thought to be associated with fragmented cement that triggered osteolysis, and was called "Cement Disease." A number of authors have reported the high incidence of periprosthetic femoral osteolysis at more than five years post-operatively (Goetz et al. 1994, Kim et al. 1993). These lesions have been attributed to high-localised stresses (Carlsson et al. 1983), stress shielding (Pritchett 1995) and micromotion between cement and bone (Huiskes and Nunamaker 1984, Scott et al. 1985).
Willert and Puls (1972) originally proposed that wear debris, generated at the articulating surfaces, during the clinical life of a joint replacement, may cause osteolysis and subsequent loosening. Debris is also produced by abrasions of the stem/cement/bone interfaces in the medullary canal, as well as by fretting at the spigot-head junction in modular components.

The debris is 'processed' by the immunological mediators of the host. Proportions of these particles are transported via the lymphatics to lymph nodes (Langkamer et al. 1992, Case et al. 1994, Shea et al. 1996). Other particles remain within the joint space causing third body wear, or reside in the synovium potentiating synovitis. The remaining particulate debris migrates to the prosthetic interfaces.

Several authors have assessed the quality of THR in which pain and functional ability were emphasised (Charnley 1979, Hierton et al. 1983, McCoy et al. 1988). Younger patients benefited from less pain with more mobility but lost more bone; less bone-stock was lost in lower mobility patients. There were reported rates of up to twice as much UHMWPE wear in younger more active patients (Charnley and Halley 1975).

A simple theoretical analysis indicates the actual wear burden on the hip joint. For a hemispherical articulation in a 28mm socket, there is 1230.88mm² of polyethylene in contact with the femoral head:

\[ \text{Area of hemisphere} = \frac{4\pi 14^2}{2} = 1230.88 \text{mm}^2 \]

The larger particles, if spherical (which they are not) and with a radius of 0.025mm, have a volume \[ \frac{4}{3} \pi (0.025^3) = 6.5417 \times 10^{-5} \text{mm}^3 \]
At low wear rates of 0.08mm/yr. (Charnley and Kamanger 1969), the total volume of polyethylene wear per year:

\[ = 1230.88 \times 0.08 \quad = 98.4704 \text{mm}^3 \]

No. of largest particles at the lowest wear rate \[ = \frac{98.4704}{6.5417 \times 10^5} \]

\[ = 1.50527 \times 10^6 \text{ per year} \]

No. of particles over 15 years \[ = 2.2579 \times 10^7 \]

If the calculation is repeated for the smaller particles at the highest wear rates, the value is \[ 6.1741 \times 10^{13} \text{ particles per year} \], producing \[ 9.2611 \times 10^{14} \text{ particles in 15 years} \]. It is difficult to appreciate the magnitude of these numbers. Seedhom et al. (1985) proposed that a relatively active THA patient takes 1.8 million steps per year. The calculated number of particles will lie between 1 and 34.3 million per step. Since the assumption that particles will only be 50\(\mu\)m is untrue, the actual numbers of particles produced per step are likely to be in the millions. This excludes the additional factors of metal and cement particles. The body is thus presented with a large amount of wear debris.

Schmalzried et al. (1992a) analysed 34 hips. Macrophages were located in the periprosthetic region, containing particles of polyethylene, many of which were under 1\(\mu\)m. Focal concentrations of macrophages containing polyethylene, metal and cement particles, were found in direct association with areas of bone lysis. Witt and Swann (1991) noted a proliferative membrane at the bone-cement interface, containing titanium and polyethylene particles, inducing a foreign-body/giant cell reaction.
Goldring et al. (1983) described a thick synovial-like membrane in loose hips at the bone-cement interface, with the ability to produce large amounts of prostaglandin $E_2$ and collagenase. Howie et al. (1988) inserted a polymethylmethacrylate plug into the distal femur of rats. After surgery particles of polyethylene ranging in size from 20 to 200 µm were injected into the joint space of the test group. Following repeated injections, resorption of bone occurred at this aseptic, non-loaded, interface. Goodman et al. (1998) reviewed studies regarding particulate debris.

Huddleston (1988), defined radiographic cystic lysis as, "a well described, localised lucency, absent on the post-op films taken immediately after the preceding total hip operation, developing at the bone-implant interface, and showing progression and unequivocal erosion of the endosteal cortex." He found that cyst formation tended to occur more distally around the prosthesis. In some cases the cysts enlarged, resorbing all of the cortical bone up to the periosteum and leaving only a thin sub-periosteal layer of bone. Often the periosteum was elevated and distended, but not broken. Several independent cysts started almost simultaneously in the periprosthetic region, progressively coalescing with each other. Resorption also occurred away from the implant by extension of the resorbing zone into healthy bone. 28 of the 42 hips had visible fractures adjacent to cyst formation (Huddleston 1988) with more described as "probably present but too small to be seen on x-ray." Most of these hips, showed measurable wear on the acetabular components. The conclusion for these hips without the normal mechanical function of the femur, is subsequent complete fracture (Pazzaglia and Byers 1984).
1.7 FEMORAL IMPLANT FAILURE MECHANISMS

Femoral loosening is thought to be initiated by debonding at the cement-metal interface (Fornasier and Cameron 1976, Stauffer, 1982, Jasty et al. 1991). Cracks occur through pores in the cement. At the outset, these are demonstrable as adjacent focal areas of lysis, caused by cement fragments, often without looseness. The cement-bone bond remains intact during the initial degeneration of the cement-metal interface. Debonding starts proximally and at the tip, extending to the mid-point region. During loading, stem pistoning occurs, resulting in the formation of metal and cement debris, which is subsequently forced through any defects in the cement mantle.

The metal-cement debonding, cement crack propagation and subsequent cement-bone debonding creates a continuous space with the joint capsule. This has been termed the "effective joint space" (Schmalzried et al. 1992a) and joint kinematics pump particulate-laden fluid around this system, facilitating wear particle mediated osteolysis.

The cement fragments noted by Harris et al. (1976), were originally thought to be the cause of the so-called "cement disease" and were actually a radiographic manifestation of the formation of wear-particle conducting channels in the cement mantle. The cracks were contributing to the cause, but not specifically the cause per se.
1.8 ACETABULAR IMPLANT FAILURE MECHANISMS

Schmalzried et al. (1992b) showed that trabecular bone adjacent to cement was resorbed due to osteolytic mechanisms along the cement-bone interface. Particulate debris generated from the articulating part of the cup is dispersed into the synovial fluid. The particles can enter small defects of the exposed bone-cement interface, or directly onto bone, leading to localised resorption. This ‘cutting wedge’ of bone resorption, which was initially limited to the circumference, forms a space into which more particulate debris laden fluid can enter, causing further bone resorption towards the apex of the cup.

The result is a fibrous membrane interposing the cement and bone. It is characterised by polyethylene debris as well as active macrophage mediated bone resorption. This membrane appears as radiolucency, the hallmark of loosening. Schmalzried et al. (1992b) also showed that the mechanical stability of the implant was inversely related to the extent of bone resorption and fibrous tissue formation.

1.9 THE CELLULAR RESPONSES TO PARTICULATE DEBRIS

An aseptically loose implant is encapsulated by an ordered synovial lining with deep fibroblastic cells, surrounded by a matrix of type IV collagen, fibronectin, laminin, heparan sulphate, entactin, type V collagen, chondroitin and keratin sulphates, with an overlying layer of macrophages (Pollack et al. 1990, Revell and Lalor 1990). This periprosthetic tissue is important in the body’s recognition of the implant (Santavirta et al. 1998).
In 1951, Newman and Scales appreciated that the response of the macrophages was dependent on the particle size (The table below summarises the type, size, and cellular location of CoCr and polyethylene particulate debris with a reference example).

<table>
<thead>
<tr>
<th>Particle</th>
<th>Size(μm)</th>
<th>Cellular Location</th>
<th>Reference Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoCr</td>
<td>1-5</td>
<td>Intracellular within large macrophages</td>
<td>Johanson et al. 1986, Boss et al. 1990.</td>
</tr>
</tbody>
</table>

Table 1.2. Cellular Distribution of Particulate Debris

Normal immunological response of the body to small particulate and foreign materials is cell-mediated assimilation, or phagocytosis. The cells responsible for this process are of the monocyte-macrophage lineage. Small particulate debris is found within macrophages. If the particle is too large for phagocytosis (>25μm), macrophages can fuse together and form a multi-nucleated giant cell that surround the particle. The precise role of the monocytes, with respect to joint prostheses is not known, but they were thought to mediate sensitivity reactions (Lalor et al. 1991). The cell reaction is related to the particulate type (Kadoya et al. 1997).
Within normal remodelling there is ongoing formation and resorption of bone. The cell responsible for usual bone resorption is the osteoclast. This large multi-nucleated cell, with its “ruffled-border” is often located at the tip of a resorption wedge. Its features are well reported (Gothlin and Ericsson 1976, Chambers 1980, Teitelbaum 1993, Athanasou et al. 1996). Osteoclasts are related to the same stem cells of the monocyte-macrophage lineage.

Cells in the tissue layers at the implant interfaces have been found to contain potent osteolytic factors (Jiranek et al. 1993, Chiba et al. 1994, Goodman et al. 1997), including Interleukin1 (IL-1), Interleukin 6 (IL-6), (Gowen et al. 1983, Stashenko et al. 1987, Herman et al. 1989, Al-Safar and Revell 1994, Westacott et al. 1992), Tumour necrosis factor-α, (TNF-α) also named Osteoclast activating factor, (OAF) (Bertolini et al. 1986, Beezhold et al. 1989, Pfeilschifter et al. 1989, Algan et al. 1996, Hicks et al. 1996), as well as various oxide radicals (Hukkanen et al. 1997 and 1998), matrix metalloproteinases (Takagi et al. 1998), and hydrogen peroxide (Kossovsky et al. 1991). These are secreted by fibroblasts, endothelial cells, and macrophages. OAF is largely secreted by macrophages.

The macrophage can be “activated” into further macrophage recruitment, phagocytosis, and the release of osteoclastic factors, by particulate debris, bacteria, and cell death. These factors proceed to stimulate osteoclastic mediated bone resorption (Murray and Rushton 1990, Roodman 1993).

Wang et al. (1996) showed that osteoclasts can phagocytose wear particulate of varying sizes. Following phagocytosis they remain fully functional, hormone responsive, bone-resorbing cells.
Chapter 1

Not only are osteoclasts capable of bone resorption but macrophages may also have an ability for bone resorption directly (Mundy et al. 1977, MacArthur et al. 1980, Santavirta et al. 1990, Kossovsky et al. 1991, Athanasou et al. 1992, Quinn et al. 1992, Lassus et al. 1998). Direct enzymatic degradation of bone is also significant in joint loosening (Hukkanen et al. 1997).

1.10 THE ROLE OF MICROMOTION

Aseptic loosening is not wholly attributed to the action of wear particles. The lack of mechanical integrity of prostheses is significant in the aetiology of loosening (Clarke 1990, Freeman and Plank-Bordeneuve 1994, Kärrholm et al. 1994, Walker et al. 1995). The maintenance and possible proliferation of a soft tissue membrane inter-posing the implant-bone interface may be attributable to micromotion (Aspenberg et al. 1992, Søballe et al. 1992, Goodman 1994). Experimentally the role of micromotion in the sensitisation for subsequent cell mediated bone lysis has been postulated (Goodman et al. 1995, Aspenberg and Herbertsson 1996).

There is also a compelling theory that fluid pressure, and not necessarily wear debris, may have a role in the loosening process (Van der Vis et al. 1997 and 1998, Aspenberg and Van der Vis 1998a&b). It is clear that there is a complex interaction of both biological and mechanical factors that eventually lead to osteolysis, associated with joint arthroplasty.
Chapter 1

1.11 THE HYPOTHESES AND THESES FORMAT

In summary, localised radiolucencies at the bone-cement interface represent loosening and subsequent pain, necessitating revision surgery. These points of focal osteolysis are caused by wear particles at the interfaces. If they can be prevented from reaching the interfaces, wear debris induced osteolysis will not occur. The proposed method to prevent migration of the particles is to attach a custom made, flexible, osteopromotive e-PTFE GORE-TEX® membrane to the implant and bone, as illustrated in figure 1.1

Figure 1.1. Schematic for Membrane Placement
Dividing the hypothesis into a number of experimental questions will test its attainability. Chapter 2 summarises the biocompatibility of the glue to attach the membrane by *in vitro* assessment. Chapter 3 will describe the osteopromotive qualities of the membrane in two rabbit experiments. *In vitro* production of wear particles will be assessed in Chapter 4 so that an animal model can be developed with accelerated wear of the acetabular cup. The objective of chapter 4 was not only to determine the effect of roughening the femoral head on the wear rate but also to monitor the morphology of the particles produced. The ability of the membrane to prevent wear particle migration will be investigated in a series of goat studies accounted in Chapter 5, with a discussion and summary in Chapter 6.
1.12 THE CONCEPT OF GUIDED TISSUE REGENERATION

The concept of mechanically sealing off a specific anatomical site for improving the healing of a particular tissue type began in the 1950s, for neural tissue (Campbell and Bassett 1953) and spinal bone applications (Hurley et al. 1959).

Much of the modern work into Guided Tissue Regeneration (GTR) has been performed in the field of periodontology, a branch of dentistry concerned with the tissues of the gingiva, periodontal membrane, alveolar bone and cementum that support and attach the teeth. An example of how GTR can be used is demonstrated below in the treatment of periodontitis.

1.12.1 A Periodontological Example of Guided Tissue Regeneration

There are substantial changes to the tooth root surface following periodontitis. The normal root is rich in collagen connecting to the adjacent bone. These can be destroyed by plaque induced inflammation, causing down-growth of the surrounding epithelium, exposing the root surface to the periodontal pocket and oral environment.

Following bacterial penetration, the root surface becomes toxic and unsuitable for the new connective tissue attachment required for regeneration. Having thoroughly debrided the site, only periodontal cells growing coronally will restore the fibre and cementum network, (Karing et al. 1985).
After surgery the periodontal ligament cells are prevented from migrating over the wound surface adjacent to the root by the dentogingival epithelial cells, which forms a long junctional epithelium thus preventing any new functional attachment. If the cells of the periodontal ligament are guided correctly, they can become established on the root surface, forming a fresh attachment provided they are isolated from the other tissues during healing (Dahlin et al. 1988).

Membranes are used to isolate tissue types over engineered defects to prevent unwanted cells accessing the site. Normally the gingival crestal fibres insert onto the cementum on the tooth surface creating a barrier thus inhibiting epithelial migration (Winter 1974). This was termed ‘contact inhibition’ and it was inferred that the connective tissue attachment to a porous biomaterial provided a similar function as collagen fibres that insert into the cementum of the tooth. Without this connective tissue attachment, the surrounding epithelium forms a sinus tract and isolates the implant, causing the material to extrude from the tissue (Robinson and Daly 1980).

Membranes unable to support viable tissue ingrowth become exposed, pocketed by endothelium, cause further tissue inflammation and poor regenerative results. Not only does GTR have a role in treating biological phenomena in disease, but also its role, as Guided Bone Regeneration, for the enhancement of orthopaedic implant osseointegration is yet to be harnessed.
Chapter 1

The subset of Guided Tissue Regeneration relevant to this work, is Guided Bone Regeneration (GBR). Much of the work into osseointegration using GBR has been performed in the field of Implant Dentistry to replace lost or missing teeth in fully and partially edentulous patients. Major contributors to the knowledge of ‘Guided Bone Regeneration in Implant Dentistry’ have compiled their experiences, in a book of the same title (Buser et al. 1994).

1.13 GUIDED BONE REGENERATION A HISTORICAL PERSPECTIVE

It will be demonstrated that the most critical issue for facilitating guided bone regeneration is the maintenance of a space, into which osseogenesis can occur. This fact was appreciated as early as 1947, by Atle Berg, who hypothesised that osteogenesis in the spine was achievable, if the paraspinal muscles were elevated from decorticated laminae using bone grafts. The orientation of bone into a particular site was conducted in the 1950’s (Hellstadius 1950, Murrey et al. 1957). Arvid Hellstadius tested Berg’s theory by using stainless steel cups and rings to raise soft tissues from the roughened cortex of rabbit femora to provoke osteogenesis.

In 1957 Murrey realised that the three critical factors needed for new bone growth were (1) adequate vascularity, (2) osteogenic cells adjacent to the defect and (3) “contact with living tissue.” To this end he protected a blood clot with a fenestrated cage. No indication was given to the fenestration size. The clot eventually filled with bone. No histological investigations were undertaken and hence the cellular activities were not known. In another study, a polyethylene tube filled with a blood clot and autogenous cancellous graft was able to bridge a 15mm gap in a dog fibula (Linghorne et al. 1960).
Melcher and Dreyer (1962) protected a blood clot containing bone defect, with either a plastic or organic shield, and found that the haematoma boundaries determined the size of the subsequent bone. The bone only formed if non-osteogenic cells were excluded from the haematoma site.

Bassett et al. (1961) and Bassett (1964) investigated the source and regulation of osteogenesis. The use of membranes for augmenting bone growth was first studied by Boyne and Mikel during the 1960s, using Millipore filters (Boyne 1964, Boyne and Mikel 1968). Placement of barrier membranes in direct contact with the outer cortical bone prevented the ingrowth of fibrous connective tissue into the bone defects.

A space was needed between the barrier and mechanically stable bone surface for a successful result. In the early 1980s this principle was used in periodontology for guided tissue regeneration discussed in the previous example. The use of e-PTFE as an osteopromotive membrane was becoming apparent (Scantlebury 1993).

1.13.1 The Replacement of Bony defects

Many lesions distort the structural integrity of bone. Regenerative treatments still prove to have complications. Lesions include many types of benign and malignant tumours, such as the fibrous dysplasias, osteosarcoma, chondrosarcoma, histiocytic tumours, multiple myeloma as well as the bone cysts. Methods for replacement can be broadly divided into biological and synthetic, as summarised in table 1.3.
Chapter 1

Biological Complications

<table>
<thead>
<tr>
<th>Biological</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autogenous (Patient derived)</td>
<td>Harvest morbidity. Limited graft supplies</td>
</tr>
<tr>
<td>Allogenic (fresh, frozen, lyophilised, demineralised)</td>
<td>Immunological, late resorption with associated fracture, cross-infection</td>
</tr>
<tr>
<td>Xenografts (Inter-species)</td>
<td>Antigenicity</td>
</tr>
<tr>
<td>Marine Coral</td>
<td>Fixation</td>
</tr>
</tbody>
</table>

Synthetic Complications

<table>
<thead>
<tr>
<th>Synthetic</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Sulphate</td>
<td>Resorption</td>
</tr>
<tr>
<td>Polymethylmethacrylate</td>
<td>Fragmentation, with subsequent wear</td>
</tr>
<tr>
<td>Polysiloxanes</td>
<td>Chronic immunological reactions</td>
</tr>
<tr>
<td>Metals</td>
<td>Wear and fixation</td>
</tr>
<tr>
<td>Ceramics</td>
<td>Weak in tension and shear, fixation</td>
</tr>
</tbody>
</table>

Table 1.3 Options for Replacement of Bone Defects

Specific substitute materials have been reviewed (Hanft et al. 1995). De Novo, host bone would be the ideal replacement for defects. This was exemplified by the Ilizarov technique for Transosseous Osteosynthesis (Ilizarov 1991). The methodology was the controlled distraction of bone fragments, at a rate that allows inter-fragmentary bone formation, without consolidation. Moreover this prevented soft tissue ingression between the bone edges, but permitted further osteogenesis as the fragments were distracted. The techniques of this practice have been classified and reviewed (Yaszemski et al. 1996, Tsuchiya et al. 1997, Yasui et al. 1997).

It is apparent that bone has an intrinsic ability to regenerate if assisted appropriately.
1.14 THE BIOLOGICAL BASIS FOR GUIDED BONE REGENERATION

Bone is a complex and diverse tissue and has been characterised both macroscopically and histologically (Hall 1991, Soames 1995). 'Regenerate' is defined as "bring or come into renewed existence." Physiological regeneration involves an ongoing replacement of tissues, typical of a normal continual metabolic process. A description of basic physiological bone regeneration or turnover has been described (Hall 1991). Reparative regeneration replaces injured or pathological tissues. If this reparative regeneration is engineered, it becomes guided bone regeneration. A basic appreciation of the biology of bone regeneration in this context is critical in the planning of surgical procedures. This will enable the creation of the environment most conducive for de novo osteogenesis.

Hence the three fundamental stipulations for bone growth are:

1. Local presence of bone forming cells, osteoblasts.

2. Ample local blood supply.

3. Mechanical support and the maintenance of space into which the osteoblasts can proliferate.

The mechanical support and the maintenance of space modulates guided bone regeneration. This determines the success of bone regeneration, which is dependent on the first two stipulations.

The repair of bony defects is a common model for the investigation of bone healing (Kaban et al. 1981, Schmitz et al. 1986, Dahlin et al. 1988, Sandberg et al. 1993). It is important to differentiate between defects where there is a physical gap interposing the bony ends, or a unicortical deficiency.
Johner (1977) examined the healing of holes in the tibia of rabbits. Hole diameters between 0.1 and 1 mm were selected; 0.2 mm diameter holes filled with lamellar bone concentrically. For holes up to 1 mm, a scaffold of woven bone is deposited over the defect and lamellar bone is deposited in the newly formed intertrabecular spaces in a 'matter of days' (Schenk and Willenger 1977). This applies to defects circumferentially surrounded completely by bone. If only one surface is bone, and the other biomaterial, spontaneous repair sizes do differ.

Another essential factor for bone formation is stability. The presence of micromotion distorts any perceived values of critical size defects at which spontaneous bone healing will take place. Harris et al. (1983) demonstrated that for an uncemented acetabular cup in a dog, a gap of 0.5 mm could not be bridged by bone, leaving a fibrous tissue envelope.

14.1.1 Critical Size Defects and Non-Union

In any discussion of bone repair an appreciation of the critical size defect (CSD) is essential (Hollinger and Kleinschmidt 1990). Schmitz and Hollinger (1986) described the CSD as "The smallest size intraosseous wound in a particular bone and species of animal, that will not heal spontaneously during the lifetime of the animal." However this is not a constant value but dependent on species, site, age, and soft tissue involvement.

For reparative processes within the CSD, guided bone regeneration resembles the process of fracture repair with haematoma formation and organisation, osteoblastic woven bone formation followed by lamellar deposition (Bhumbra et al. 1998).
Bony union will not occur, without assistance, for defects beyond the CSD. Other factors can also cause bony non-union, even if the defects are within the critical size, such as instability, periosteal disruption, interposition of soft tissue, infection, inadequate vascularity, as well as nutritional and metabolic alterations (Mathog and Boies 1975, DeChamplain 1973).

For even larger defects, methods for aiding regeneration are required. This is achieved by providing a scaffold to allow cellular colonisation and integration. Analogous with the ‘race for the surface’ between host cells and bacteria proposed by Gristina (1987), as a subset response from the host tissues there exists a further “race”.

Guided Bone Regeneration protects a space into which the normal processes of bone regeneration can occur (Dahlin et al. 1991). The need for this space is defined by the other tissues’ ability to occupy that site at a more rapid rate. Mechanisms that regulate this process are not clear. In normal bone any breach in the mechanical integrity will activate local bone regeneration by the release of the growth factors, Insulin-like Growth Factor (IGF), Transforming Growth Factor (TGF), Fibroblastic Growth Factor (FGF), as well as Platelet Derived Growth Factor (PDGF). Other bone inducing factors include Lacroix’s (1947), osteogenin, and the Bone Morphogenetic Protein Family (Urist and McLean 1952 & Urist et al. 1967). The presence of the membrane may allow these factors to concentrate their effects as well as remaining in location for longer periods of time.
Ogiso et al. (1991) showed in vitro that fibroblasts produce one or more soluble factors that have a deleterious effect on bone stem cell differentiation and osteogenesis. Schmitz et al. (1990), proposed that in the absence of appropriate bone derived augmentation and specific growth factors in large defects, cells are unable to differentiate into osteoblasts and to calcify the matrix. It is clear that the role of the osteogenic stem cell and the stromal system of bone and marrow is essential (Beresford 1989).

Obviously orthopaedic practice is not only concerned with bony defects. As discussed previously, the treatment of joint disorders is also an extremely relevant and critical issue. The complete incorporation of orthopaedic implants working symbiotically with the body is a goal yet to be fully realised.

1.14.2 History of Osseo-Integration

The major step of what is now called osseo-mechanical integration (Walker and Blunn 1997) was taken by Brånemark et al. (1969). Osseomechanical integration is the formation of bone, up to the implant which in combination with stresses engineered by the design, maintain and remolds bone, such that the implant becomes fully integrated into the skeleton. Schroeder et al. (1976) provided histological evidence of direct bone-titanium contact, in an undecalcified section of a dental screw. In 1977 Brånemark used the term 'osseo-integration.'
These implants have had long-term follow-up (Adell et al. 1981 & 1987), with a survival rate of 86% in the mandible and 78% in the maxilla. These and other positive survivorship figures from fully (Babbash et al. 1986, Bruggenkate et al. 1990) as well as partially edentulous patients (Buser et al. 1991, Zarb and Schmitt 1993a&b), widened the scope for patient selection.

Patients presenting with equivocal indications such as recipient sites without sufficient bone, sites in the locality of specific anatomical structures (mandibular nerves, maxillary sinus, nasal cavity) extraction sockets, and high-demanding aesthetic results became candidates for treatment consideration, with new Guided Bone Regenerative technologies.

1.15 EXPANDED POLYTETRAFLUOROETHYLENE (e-PTFE)

Fluorocarbons are generally very stable compounds. PTFE is produced from tetrafluoroethylene which in turn is produced from the fluorination of trichloromethane.

PTFE is an extremely long carbon chain protected by a dense sheath of fluorine, \((\text{CF}_2-\text{CF}_2)_n\), making it inert to host responses. The polymer is mixed with lubricant, naphtha, to form a Teflon® paste that is shaped under high pressure. This material is made porous by a novel mechanical stretching process and the porosity controlled by sintering at high temperatures. Pore size and shape is modified by modulating the processing conditions and the post-sintering cooling rate. Forms of the e-PTFE Gore-Tex® prostheses have an additional thin external film of highly orientated PTFE.
Host responses cannot chemically react with the densely fluorinated carbon chain, hence the material is accepted and tissues continue to undergo a healthy turnover. The e-PTFE membrane has been shown to provoke an inflammatory response that is slightly more intense than a sham operation (Lam et al. 1995).

The aforementioned property of 'osteopromotive' is an index of the proliferative ability of bone, given the correct environment. e-PTFE is chemically able to provide this environment by being so inert as to not provoke a chemical response. However it does show a very high water contact angle, defining e-PTFE as a highly hydrophobic material (Lam et al. 1995).

e-PTFE is a three dimensional matrix of nodes and interconnecting fibrils. The material elicits a similar response when implanted into different sites, with encapsulation and cell colonisation (Béllon et al. 1996). However the membrane can be produced with different porosities that in turn alter the host responses in certain environments (Hirabayashi et al. 1992). Figures 1.2, 1.3, and 1.4 are scanning electron micrographs of the membrane surface. Figure 1.2 was taken at a low magnification, showing the nodes and fibrils. Figure 1.3 demonstrates the nodes and inter-connecting fibrils, at a higher power. Figure 1.4 was taken through the section of the membrane and illustrates the two porous layers, with the inner occlusive layer.
Figure 1.2 Photoelectronmicrograph of e-PTFE surface. Low Power

Figure 1.3 Photoelectronmicrograph of e-PTFE surface at higher power
e-PTFE has a long history in implant density. The use of this particular material is not requisite for guided bone regeneration, and there are other membranes that can function within this context (Kleinschmidt et al. 1993, Sandberg et al. 1993, Ashammakhi et al. 1995, Zellin et al. 1995, Piatelli et al. 1996, Pineda et al. 1996).

For guided bone regeneration the membrane should be biocompatible, occlusive to cells but not fluids, have the structural integrity to form a ‘space’, allow tissue ingrowth hence stability, and be clinically manageable (Hardwick et al. 1994). e-PTFE has the longest experimental record in guided bone regeneration. It has been shown that membranes constructed of different materials vary considerably in osteopromotive efficacy (Zellin et al. 1995).

In 1988 Dahlin et al. used expanded polytetrafluoroethylene (e-PTFE) as the ‘barrier membrane’ to test the GTR principle with GBR. Having lifted mucoperiosteal flaps, 5mm holes were drilled bilaterally through the mandibles of 30 dogs. Controls had defects that where covered by just the mucoperiosteal flap, and the test sites had e-PTFE membranes (W.L. Gore and Associates, Flagstaff, Arizona) placed over the defects, prior to flap coverage and wound closure. Histomorphometric and gross analysis showed that the control defects were filled with fibrous connective tissue with a slight ingrowth of new bone at the defect margins whereas the test groups demonstrated new complete bone regeneration. Hence following further testing (Dahlin et al. 1990, Seibert et al. 1990) the GBR principle became clearer.
Dahlin (1993) used $^{3}\text{H}$ thymidine as a bone marker in rats. Ten days after surgery, endosteal cells within the bone adjacent to the membrane defects were undergoing mitosis. Incorporation of $^{45}\text{Ca}$ showed that test defects had a significantly higher quantity of mineralisation, even though the rate of uptake was slower. Maximal levels were reached after five weeks in the test specimens and two weeks in the controls. In rats, the potential for osteogenesis is high and so the rate of bone formation would be slower in man (Schmitz et al. 1986).

Linde (1993a) showed the osteopromotive effect of e-PTFE membranes can also cause bone to grow in anatomical sites where ordinarily bone is absent. He used a stiff dome-shaped material placed on top of the flat calvarium of rats, forming a sealed space into which osteogenic cells could migrate. The establishment of a blood clot beneath the membrane into an actual space is of paramount importance in allowing predictable osteogenesis. This study used a very porous form of e-PTFE, with 100$\mu$m pores, which also allowed angiogenesis. Moreover bone formation was also seen outside the membrane.

The variation in pore size and the ability of neovascularisation has been investigated (Brauker et al. 1995). Results suggest that larger pore diameters are able to support vascularisation by allowing cell entry.

With the rapid increase in awareness of the bone/e-PTFE interactions work into the applications of guided bone regeneration has spread into other fields, albeit slowly (Ashammakhi et al. 1995, Nyman et al. 1995, Piatelli et al. 1996, Bhumbra et al. 1998). Nyman et al. (1995) concluded it was possible to use the principles of GBR to achieve bone union of segmental long bone defects.
**Osteogenic** cell origin is important, in aiding their migration and proliferation. In fractures, the cells derive from the periosteum and marrow. Under the defect, cell recruitment arises mostly from the endosteum, as well as from Haversian and Volkmann canals.

### 1.16 PRACTICAL ASPECTS OF GUIDED BONE REGENERATION

The maintenance of a ‘space-making site’ is essential, and failure to make provision for this results in failure of GBR (Dahlin *et al.* 1988 & 1989, Melcher and Dreyen 1962, Seibert and Nyman 1990, Kohavi *et al.* 1991). This is often the case when implants are placed in areas surrounded by inadequate bone volume such as dehiscence defects, cartilage fenestration defects or residual intraosseous defects.

For acceptable results, a defined, stable region into which bone is intended to grow during healing must be created and maintained for a sufficient period of time. Bone regeneration in membrane treated periodontal defects in dogs, is a *function of the amount of space available* (Haney *et al.* 1993).

To maintain an adequate space within the desired location, the membrane must be able to support its own weight as well as sustain the pressures exerted by the presence and possible movements of overlying tissues. Membrane collapse with the disruption of space will prevent bone formation; hence a degree of membrane stiffness is essential.
The contours of new bone are defined by contours of the membrane boundaries (Kohavi et al. 1991). However, the membrane cannot be too stiff, as this reduces its adaptability to the surfaces to which it is attached. An added value of stiffness is the ability for the membrane to have a degree of 'memory' to the surface contours.

Membrane collapse into the space can be prevented using a scaffold, or filler. A collagen sponge (Collagen Fleece, Pentapharm AG, Basel, Switzerland) has been used (Buser et al. 1994) under membranes to secure a blood clot in the bone defect. It is used in conjunction with supporting screws to elevate the membrane since the collagen sponge cannot support the membrane solely.

Alternatively autogenous bone graft provides an excellent potential for neo-osteogenesis. This can be stabilised by the injection of intravenous blood to provide a scaffold for bone formation, analogous to fracture repair.

Having defined and maintained the space, the priority shifts to membrane fixation, with subsequent immobilisation. Movements of the membrane have deleterious effects on the bone formation. Micromotion of many surgical prosthetic devices leads to the formation of encapsulating fibrous tissue. This has been demonstrated in hip replacements (Aspenberg et al. 1992, Søballe et al. 1992, Goodman 1994) as well as dental implants (Brunski et al. 1979). Membrane movements compromise the bony contact, permitting soft tissue ingrowth.
Fixation techniques, for accurate membrane placement in dental applications, include cover screws as well as stainless steel mini fixation screws (Schenck et al. 1994). These devices also facilitate radiographic membrane location and were based on mini-screws used for bone fragment stabilisation in maxillofacial surgery. They have been specifically modified for membrane placement (Memfix System, Institute Straumann AG, Waldenburg, Switzerland).

There remain a number of different methods for membrane attachment, including standard sutures (Dahlin et al. 1988). In some cases the sutures themselves have been made of e-PTFE (Linde and Hedner 1995, Zellin et al. 1995). Reinforcement with e-PTFE rings has also been used (Nyman et al. 1995). Common membrane attachment appears to be by press-fit placement with further support and coverage from the local periosteal flap (Nyman et al. 1990, Linde et al. 1993, Piatelli et al. 1996).

The issue of membrane attachment is an important one. In most of the dental applications of GBR there are two relevant differences. (1) Dentists have the luxury of membrane placement in a stable, non-kinematic bearing environment. (2) The e-PTFE membrane is used for guiding bone formation, and then is subsequently removed. It is intended that the membrane will remain indefinitely in situ for our application. These differences relative to the orthopaedic joint environment may necessitate a modification of attachment technique.
For membrane attachment on both the femoral and acetabular sides there is an inherent lack of congruent surfaces, albeit more so in the former. It is absolutely essential to ensure direct e-PTFE-bone contact in order to reduce the likelihood of soft-tissue encroachment. Rigid fixation avoids the entrapment of the membrane within the articulation.

The attachment method that proved most feasible and fulfilled the above criteria was the use of a biocompatible butyl-cyanoacrylate adhesive.

1.17 THE USE OF BUTYL-CYANOACRYLATE ADHESIVE

Cyanoacrylate have been used experimentally and clinically for decades as a tissue adhesive as well as a haemostatic and embolic agent (Awe et al. 1963, Orda et al. 1974, Vinters et al. 1985).

In 1949, the cyanoacrylates were discovered and they were first reported as adhesives by Coover et al. (1959). Liquid in monomer form, they polymerise rapidly with an exotherm upon exposure to water. They are made from cyanoacetate and formaldehyde, and changing the initial alkyl group can alter the chain length. This alkyl group has consequences for biocompatibility and subsequent degradation by hydrolysis (Pani et al. 1968). Methyl-2-cyanoacrylate was used widely in the initial stages, but was abandoned when recognised to have a degree of histotoxicity (Leonard 1968). A reduction in the acute inflammatory response, with an increase in polymerisation time was noted when the alkyl chain was lengthened. The optimal preparation to date characterised by a four-carbon chain, is butyl-cyanoacrylate. This is available in both N-butyl and Iso-butyl cyanoacrylate forms. Both have been used, but the latter more so.
N-Butyl-2-cyanoacrylate is biocompatible and resorbable (Reynolds et al. 1966, Pani et al. 1968). The cyanoacrylates are degraded and excreted in urine (Reynolds et al. 1966). The most common and widely used application of the cyanoacrylate is for the closure of superficial skin lacerations, especially in children (Gahl et al. 1984, Morton et al. 1988, Mizrahi et al. 1988, Watson 1989 Applebaum et al. 1993, Quinn et al. 1993, Vobel et al. 1993). Its also has been shown to have a degree of bacteriostatic action (Howell et al. 1995).

There are a variety of formulations available although Histoacryl Blue® (B.Braun, Melsungen AG) is the most widely used in the clinical setting. Histoacryl Blue® is supplied in 0.5ml plastic vials, in boxes of 5. The monomer contains a dye, 1-hydroxy-4-p-touidion-antrachion that imparts a blue appearance making it clearly visible in the surgical field. It is applied using a fine plastic nozzle although an aerosol has also been utilised (Quillen and Rosenwater 1994).

Complications of its use include exothermic liberation, and a quick setting time of 20 seconds, thereby not permitting tissue replacement. It also has a lack of tensile strength relative to sutures (Bresnahan et al. 1995).

As well as dermatological applications the cyanoacrylates are also used for deeper tissue applications, including bony repair of the cranium (Amarante et al. 1995) or the appendicular skeleton (Capasso et al. 1991), meniscal repair augmentation (Koukoubis et al. 1995), cannalicular occlusion to treat the dry eye (Diamond et al. 1995), sealing corneal perforations (Dart 1996), cardiac surgery (Robicsek et al. 1994), and gastrointestinal surgery (D'Imperio et al. 1996).
1.18 THE GOAL

The specific goal is to apply the techniques of GBR in the prevention of loosening of total hip replacements.

A more generic goal is to introduce the guided bone regenerative concepts to orthopaedic surgery, for subsequent uses in bone and soft tissue applications.
Chapter 2 Biocompatibility and the *In Vitro* properties of e-PTFE and Butyl-cyanoacrylate Adhesive

2.1 Introduction

Our aim was to establish the *in vitro* biocompatibility of e-PTFE combined with butyl-cyanoacrylate on a human-osteosarcoma cell line. *In vivo* experimentation has traditionally followed *in vitro* screening. Osteoblast-like cells were selected since the biomaterial/bone interactions were critical in determining the guided bone regenerative capacity of the initial seal.

*In vitro* systems have been used widely for biomaterial testing (Johnson *et al.* 1985, Kirkpatrick and Mittermayer 1990, Vince *et al.* 1991, Hunter *et al.* 1995, Morrison *et al.* 1995). The greatest benefit of *in vitro* testing is the ability to control and define specific criteria and compare their relative contributions to the biological system being analysed. However, the most important shortcoming is the lack of circulatory and lymphatic systems for both the removal of harmful products and the nourishment of the cells *in situ*. It is therefore clear that in order to gain the most information about a test biological system both *in vitro* and *in vivo* experiments should be conducted.
Chapter 2

The primary objective of the study was to characterise the mitogenic effect of butyl-cyanoacrylate and e-PTFE using the HOS cell line TE85. These were obtained from the European Collection of Animal Cell Cultures (ECACC No. 87070202), and were used between passages 6 and 12 in all experimentation. The HOS cell line has a consistent reproducibility and a rapid turnover rate, yielding large cell numbers for subsequent assays. It is appreciated that larger bone in vitro studies ought to evoke the use of primary osteoblasts, since proliferation and alkaline phosphatase activity of HOS cell lines cannot be directly correlated (Clover and Gowen 1994). Nevertheless, the HOS data provides an indication of how the particular biomaterial affects the relative proliferation rates.

Proliferation was quantified using [\(^3\)H] thymidine incorporation per microgram of DNA. Cells in the S-phase take up 3H-thymidine for incorporation into newly synthesised DNA. The limitations of this method have been detailed by Maurer (1981), and these include radiochemical impurities in [\(^3\)H] thymidine, incorporation into RNA, protein and lipid fractions as well as the DNA, and the possibility that labelled thymidine may affect the structural integrity of DNA. Therefore, results drawn from such experimentation should be interpreted cautiously. However, Maurer does acknowledge that these ‘artefacts’ may be of varying significance and should be considered with respect to individual cases. Despite these shortcomings, thymidine incorporation is still used to quantify cellular proliferation (Hunter et al. 1992). We accept that for a more detailed in vitro analysis other factors such as cell morphology, rate of cell death, and other indices of proliferation ought to be considered.
2.2. Materials and Methods

2.2.1. Elution Test.
An international standard designed specifically for the biological evaluation of medical devices has been established. Method criteria determining in vitro testing for cytotoxicity have been followed as defined by Part 5 of ISO 10993-5. In the first stage of this investigation we studied the incubation of cultured HOS TE85 cells with serum extracts of porous e-PTFE, butyl-cyanoacrylate, reagent control and an organo-tin stabilised poly(vinylchloride) toxic positive control.

The extraction medium was Dulbecco's Modified of Eagles medium (DMEM; Flow, Irvine, Scotland) supplemented with 10% fetal calf serum (FCS), 1% glutamine, 2% Penicillin/streptomycin, and 2% HEPES [4-(2-hydroxyethyl)-1-peperazineethanesulfonic acid] (all supplied from Life Technologies, Paisley, UK), cultured in tissue culture plastic (Falcon, Beckton Dickson UK Ltd., Cowley, Oxon., UK). Extraction time was 24 hours at 37°C. Circulation of the extraction medium was achieved with a rollerplate. A sheet of autoclaved e-PTFE measuring 11 X 7 centimetres was used, giving 5.13 cm²/ml of biomaterial elution in a 30ml serum sample, since both sides of the membrane were 'active.' However, the e-PTFE was porous, making the actual surface area indeterminate. The above dimensions also happened to fall within the defined weight criteria, of 0.1477 g/ml. The positive control of organo-tin stabilised poly(vinylchloride) was sectioned into the same 11cm. X 7cm. sheet. The butyl-cyanoacrylate glue was exposed to an area of 79cm² by coating the walls of a Falcon centrifuge tube with the adhesive. A 30ml reagent control, with only serum, was also tested.
HOS TE85 were seeded into 2 twenty-four-well culture dishes in 0.5 ml of serum, at a cell concentration of 2 X 10^4 cells/ml at 37°C, and 5% CO₂. After 24 hours the extractants were added. For each of the test materials, six subgroups of increasing serial dilutions were tested. These were from the original extract. Then dilutions of X2, X4, X8, X16 and X32, with fresh DMEM and supplements to dilute the extract were tested. Cells were cultured in the complete supplemented medium together with 1μCi/ml of [³H] thymidine (Amersham Life Science, UK). After a further 24 hours incubation the cells were rinsed with phosphate buffered saline (PBS) at 37°C, stored in 1ml of de-ionised water and freeze thawed three times at -70°C and 37°C to ensure cell rupture. Cell proliferation was tested using radiolabelled thymidine incorporation by liquid scintillation; 100 µl of the digest was placed into 96 wells lined with 0.45µm pore-size filtration plates (Millipore, Watford, Hertfordshire, U.K.) with 20% tri-chloroacetic acid for 30 minutes at -4°C in order to precipitate the DNA. The DNA precipitate was rinsed again in TCA to remove any unbound tritium, then dried, and the filters punched out into scintillation vials. The precipitate was dissolved with 0.01M potassium hydroxide and mixed for 30 minutes. Four mls of Emulsifier Scintillator-Plus fluid (Canberra-Packard, Berkshire, U.K.) was added to each vial and mixed again for 30 minutes. Readings on the scintillation counter, Minaxi Tri-carb 4,00 series (Canberra Packard), were taken over 5 minutes each well. Results were expressed as counts per minute per ug DNA. DNA fluorimetry was performed in order to obtain absolute DNA mass, by bisbenzimidazole-Hoechst 33258 (Sigma Chemicals, UK) (Kapuscinski and Skoczylas 1977, Rago 1990, Rao and Otto 1992).
2.4.2 Cell Seeding Test

Cell growth was tested on the following biomaterials, and their combinations: (1) 5 mm Thermanox discs (Life Technologies), as the control surface, (2) a sterile silicon medical adhesive type A (Silastic®, Cat. No. 891, Dow Corning Corporation, Medical Products 1051393-0390, MI. 48640, U.S.A), (3) the butyl-cyanoacrylate (Vetbond™, 3M, No. 1469, St. Paul, MN 55144-1000), (4) e-PTFE (W.L. Gore and associates, Flagstaff, Arizona), (5) e-PTFE glued to the base of the well by silicon glue, and (6) e-PTFE glued to the base of the well by the butyl-cyanoacrylate. Cells were seeded as in the first test. e-PTFE is extremely hydrophobic and floats on water. Therefore to allow cell attachment for the culture of e-PTFE only, the volume was halved and the cell concentration was doubled. This formed a large drop over the e-PTFE surface and prevented the membrane from floating, thus allowing the cells to attach whilst in media.

After the initial 24 hours, following addition of the $[^3]H$thymidine, the e-PTFE was inverted into the fresh culture to monitor the attached cell proliferation. Proliferation quantification was as in the first test.
2.3 RESULTS
2.3.1 Elution Test
After the initial 24 hours, and before the extractant was added, microscopic visualisation (Olympus IX70) during culture demonstrated that some of the cells had attached to the tissue culture plastic well base, but they were not confluent.

Figure 2.1 demonstrates the Counts per minute divided by the mass of DNA (CPM/DNA). It shows the positive control, clearly indicating that the cells were susceptible to the deleterious effects of organo-tin stabilised poly(vinylchloride) on their proliferation in a dose-dependent manner. The propagation of HOS cells on e-PTFE and on reagent control was not statistically different from each other. The growth of cells on the butyl-cyanoacrylate appeared to be inversely related to its elutant concentration. After X4 dilution, cell proliferation had reached a level that was comparable to that of the e-PTFE and reagent control.

![Figure 2.1 Cell Proliferation in Elutant Dilution](image-url)

Figure 2.1 Cell Proliferation in Elutant Dilution
2.3.2 Cell seeding

The results shown in figure 2.2 clearly demonstrate that Thermanox was the most inductive of the seeded materials, with the e-PTFE reaching 50% of that level. The silicon glue produced cell proliferation at 25% the level of Thermanox when used on its own, as well as in conjunction with the e-PTFE.

![Figure 2.2 Cell Proliferation over Different surfaces](image-url)

Figure 2.2 Cell Proliferation over Different surfaces
2.4 DISCUSSION

Results from the first set of experiments demonstrated that there were no components, which leach from e-PTFE into serum that had an adverse effect on cellular proliferation of human-osteosarcoma cells. The second surface growth experiment showed that e-PTFE provided a where cellular proliferation relative to a Thermanox control was reduced to 50%. Reasons for this disparity are unclear. The porous surface may have had an effect, limiting the rate of proliferation. Another possibility may be that the hydrophobic surface of the e-PTFE affects the initial protein adsorption, affecting the proliferative rate relative to the Thermanox.

Figure 2.1 indicates that the positive control functioned correctly in a dose dependent manner, and that the cells were susceptible to effects deleterious to their proliferation. A similar dose dependent response was noted for dilutions 1, 2, and 4 for cyanoacrylate. It was therefore clear that factors leaching from the glue in serum had the ability to affect proliferation. It was difficult to know how the adverse effect, noted with the butyl-cyanoacrylate over 24 hours proliferation in a culture well, would actually affect the cells in vivo. In the formation of butyl-cyanoacrylate, formaldehyde reacted with cyanoacetate. Although Pani et al. 1968 demonstrated that the cyanoacrylates are degraded by hydrolysis, the rate of which is dependent on the chain length.
The dye has a blue colour, caused by the addition of 1-hydroxy-4-p-toluidion-antrachion, allowing clear visualisation in the surgical field.

For both the control and e-PTFE there were inexplicable differences in the cellular proliferation at the various dilutions as demonstrated by figure 2.1. There were no apparent theoretical reasons why these aberrant readings were present, so they can only be attributed to insufficiencies in experimental practice.

The readings from figure 2.2 demonstrate that for the initial 24-hour cell proliferation, the attachment media was the rate-limiting step. This was concluded from the equal counts per minute per DNA, of the adhesives as well as the attached e-PTFE. The value of testing e-PTFE individually was clear; so that any "combination effects" can be overcome when comparing with just the adhesive cultures. When the results from the first experiment were considered, it was likely that the same "chemical leaching" was occurring and affecting cell proliferation. Although this may be true for the butyl-cyanoacrylate, the low proliferation of the cells on the silicon glue was surprising. Silicon glue was believed to be an inert material for the attachment of various biomaterials for cell biocompatibility testing yet has been demonstrated not to be inert, when compared to controls. Obviously its role for this use merits reconsideration.

It was important to note that these results represent the cellular proliferation over the biomaterials during 24 hours. When the actual longevity of the materials within their roles was considered, 24 hours gives only a small temporal representation of the entire role of the biomaterial. Nevertheless, the results indicate that cyanoacrylate and e-PTFE reduced proliferation when compared to normal controls, for the first 24 hours.
Chapter 3 Enhanced Bone Formation and Osseointegration using Guided Bone Regeneration

3.1 Introduction The practical aspects of Guided Bone Regeneration

3.2 Materials & Methods

3.3 Results

3.4 Discussion

3.1. INTRODUCTION

As discussed in chapter 1, many studies have investigated the role of e-PTFE membranes in the augmentation of bone defects. These have been conducted in association with dental and maxillofacial work and thus have involved primarily, flat bones of the skull and mandible (Jovanovic et al. 1992, Becker et al. 1994, Dahlin et al. 1994, Hanson et al. 1994, Hedner and Linde 1995).

In a study involving bone defect size, it is important to be aware of the role of the critical size defect (Schmitz and Hollinger 1986, Hollinger and Kleinschmidt 1990). However there are bound to be variations between different bones as well as the location within that particular bone. The lateral femur of the rabbit is a common site for experimentation in defect healing investigations (Fyda et al. 1995).

When the original hypothesis is considered, it is apparent that the exclusion of wear particles from the interfaces would be markedly assisted by the formation of a biological seal, evoking the use of guided bone regeneration. The theoretical and practical basis of guided bone regeneration has been discussed in Chapter 1. This chapter reports the use of a small animal model that reproduced some of the biological principles we hoped to use in the large animal model to test the exclusion of wear particles from the bone-implant interface, using e-PTFE.
Two studies in the corticies of long bone have been conducted. The first involved two uni-cortical defects; the test defect was covered with an e-PTFE membrane and the control site left uncovered. This test was conducted to give an understanding of the biological responses for bone defect healing with guided bone regeneration. The second study assessed the role of guided bone regeneration in assisting osseointegration of titanium alloy.

We aimed to statistically quantify the bone growth into a defect, the effect of a semi-permeable membrane on the rate and stage of bone regeneration, and the effect of the membrane on the osseointegration of an implant. As the membranes are attached to the bone using the butyl-cyanoacrylate, the in vivo biocompatibility response of both materials in conjunction is also assessed.
3.2 MATERIALS & METHODS
Experimentation was conducted in accordance with the UK Animal (Scientific Procedures) Act of 1986. New Zealand White rabbits aged between 20-22 months, weighing between 3.2-4.0kg, were randomly selected for each experimental group. The prototype e-PTFE membrane had three layers. The outer two surfaces were each approximately 0.5mm thick and designed to allow tissue growth throughout those layers. There was also a middle layer, less than 1μm thick that is less permeable and allows mutually exclusive tissue types to grow throughout the outer two matrices.

Animals were sedated with 0.2mls of Diazepam followed by 2mls of Hypnorm (Fentanyl™) intramuscularly 15mins prior to surgery. Anaesthesia was maintained with Nitrous oxide and oxygen at 1:1 with a 1-2% mixture of fluorothane. An incision of 2 cm was made 2cm distal to the greater trochanter along the lateral aspect of the femur. The fascia lata was incised and the femur exposed by blunt dissection.

3.2.1. Experiment Model I.
Four uni-cortical femoral defects were drilled into each of the nine animals. Two 2mm diameter holes were drilled into the antero-lateral aspect of each femur. They were drilled into the diaphysis of the femur, each separated by 3cm. One of the two holes had a 5mmx5mm square of the e-PTFE prototype membrane glued over the defect using butyl-cyanoacrylate (VetBond™, 3M St. Paul, MN) applied to the margins of the square. The site was irrigated with saline, followed by closure of the fascia latae and skin.
Chapter 3

One rabbit suffered a two day post-operative femoral fracture, hence was subsequently replaced. The femora were harvested in 3 groups: at two weeks, one month and two months. At harvest, the vastus intermedius over the defect site was preserved. The femora were placed immediately into 10% buffered formal saline for processing by decalcified histology.

Wax embedded sections were cut using a sledge microtome in the midpoint of each of the holes, and were stained with Ehrlich’s Haematoxylin and Eosin (H&E). Sections were taken across the midpoints to allow the osteogenic potential to be assessed across the entire defect.

Osteogenesis was expected to proceed from the margins of the hole inwards. All tissues were quantified by scanning the slides using Neotech Image Grabber™ followed by image analysis with Optilab™ software. The extent of bone healing is expressed by the bone area, in the defect excluding the lacunae, in the defect as a percentage of the total defect area.

![Figure 3.1 Schematic of Bone Defect](image)

3.2.2. Experimental Model II

The second model investigated the osseointegration of titanium (6%Al,4%V) alloy screws.
Two in-line 2mm transcortical holes were drilled, that passed through the antero-lateral aspect and postero-medial cortices of the femora of Eight New Zealand White rabbits. The antero-lateral hole was enlarged to 3mm. A 11mmX2mm partially threaded titanium screw was inserted transcortically, leaving a gap of 0.5mm between the margins of the 3mm hole and the implant. For the test femora the e-PTFE membranes were glued over the defects.

After tissue fixation and dehydration the implants within bone were embedded into methyl methacrylate resin, and sections showing the mid-point of the screw were taken by measuring a maximal diameter of 2mm. Following grinding and polishing, the slides were stained with Toluidine Blue and Paragon stains. The amount of bone bridging the gaps was calculated by taking means from either side of the implant. Bone contact was expressed as a percentage of the total tissue in contact with either side of the titanium screw, adjacent to the antero-lateral cortices.

The second model attempted to reproduce a system for the larger animal series shown in figure 3.2.
Figure 3.3 is an operative photograph of the antero-lateral aspect of the rabbit femora, demonstrating the insertion of the screw without the Gore membrane covering. The photograph clearly shows the space between the bone and the edges of bone, of 500μm.

A small Langenbeck retractor is pulling the belly of vastus intermedius away from the screw head. The bone surface has been cleared with a periosteal elevator in a sham procedure to facilitate e-PTFE attachment should this have been a test group animal.

A test site, with the e-PTFE membrane attached is shown in figure 3.4. The defect was covered in a 5mmX5mm square of e-PTFE membrane, attached by butyl-cyanoacrylate glue.
Figure 3.4 Test site covered in e-PTFE membrane
3.3 RESULTS
Results from the two experiments have been subdivided into two sections for clarity.

3.3.1 RESULTS: Model I
After two weeks there was a visible increase in woven bone filling membrane covered defects (Figure 3.6) than controls (Figure 3.5). The control site contains some woven bone, but not in the quantity as the test sites. The test-site shows osteoblastic tissue entering the matrix of the e-PTFE membrane. The bone growth in the test sites is at a more advanced stage than the controls.

Figure 3.5 After two weeks in the uncovered defects there is the beginning of woven bone deposition into the space. The mid-point of the defect still contains haematoma. (X40)
Bone formation occurs within the defect site, with a visible increase of woven bone filling the membrane covered defect. At both one and two months, the control and test groups showed that bone had regenerated to bridge the defects (Figures 3.7 & 3.8).
Figure 3.7 Lamellar Bone filling Control site after two months (X40)

Figure 3.8 Lamellar Bone filling Test site after two months (X40)
Polarised light microscopy revealed immature woven bone in the defect region, in contrast to the mature lamella bone seen in the adjacent cortex. The e-PTFE membranes were integrated with fibroblasts on the antero-lateral side and with osteoblasts on the opposite side, where the membrane was adherent to cortical bone and the defect. The defect areas contained numerous cells of an osteoblastic appearance that were penetrating the matrix of the e-PTFE membrane, in retrievals from all time periods (Figure 3.8: two weeks).

The membrane was observed to have maintained its normal integrity throughout the time periods. In the two week and one month post-operative retrievals inflammatory cells were still present. These cells were not seen at two months post-operatively indicating no adverse reaction to either the membrane or the glue. The bone area in the defect contained pores, which were related to the stage of bone regeneration. As the bone changed from woven to lamellar bone the size and number of the gaps decreased in both control and test groups.

Results for bone filling the defects in the first model are summarised in figure 3.10.
Figure 3.10 e-PTFE significantly enhanced Bone Regeneration

The dotted line represents the percentage bone in the adjacent normal cortices. For both control and test groups there was a statistical difference in bone growth between all of the time periods. Using a student’s t-test, assuming unequal variances, it was shown that the defects covered with the membrane, at just two weeks, had significantly more bone (p<0.01). After one and two months, the membrane-covered defects had increased bone formation (p<0.02) compared to the controls.
3.3.2. RESULTS: Model II

In the second model, the tissue area on either side of the sectioned implant filling the 500μm engineered space was recorded. The total pore area, non-osseous tissue and fibrous tissue area were subtracted from the total area in the space. These values were expressed as the proportionate amount of bone filling the defect. Using this method, bone lacunae were not defined as bone. It is our intention to give an index on the stage of bone regeneration using this method. This was apparent by observing the original bone adjacent to the defects.

The percentage bone in contact with the implant was statistically significant at one and two months (p values<0.02) in the defects covered with membranes, as compared to the uncovered defects (Figure 3.11).

![Graph showing bone contact with implant over time](image)

Figure 3.11 e-PTFE significantly enhanced Osseointegration
The percentage bone bridging the gap between the defect and the implant was not significant at one month, but was significant at two months. (P<0.02).

Uncovered and covered defects are shown in figures 3.12 and 3.13 respectively. At one month the uncovered defects (Figure 3.12) had fibrous tissue adherent to the implant continuous with overlying soft tissue. There is no bone-growth up to the implant in these cases. At one month for the covered defects, bone has filled the engineered space and grown up to the implant. The membrane covered defects excluded fibrous tissue from the dead space after one month.

The control and test groups after two months are shown in figures 3.14 and 3.15 respectively. In the control sites, bone has grown into the space, but there is still a blue-staining fibrous tissue membrane surrounding the implant. For the test groups the membrane has prevented the formation of fibrous tissue, and the screw is osseo-integrated. This is confirmed in the higher magnification images of figures 3.16 and 3.17.

The membrane covered defects excluded fibrous tissue from a space, into which bone was guided to grow.
Figure 3.12 Section through control implant after 1 month, showing little bone regeneration into the engineered space. (X40)

Figure 3.13 Section through covered implant after one month, with Enhanced Bone Regeneration into Defect and up to Implant (X40)
Chapter 3

Figure 3.14 Section of Control Group with Fibrous Membrane around Titanium after two months

Figure 3.15 Section with Complete Bone Integration of Implant in Test Group after two months
Figure 3.16 Section with Fibrous Tissue against Titanium in Control Groups after two months (X200)

Figure 3.17 Histological section showing bone directly against Titanium Implant in Test Groups after two months (X200)
3.4 DISCUSSION

In both experimental models, the presence of the membranes led to a significant increase in bone regeneration. The second model also demonstrated increased osseointegration of titanium implants when using an e-PTFE membrane. Without the membrane covering the titanium screws and engineered defects, fibrous tissue was found to encapsulate the implant.

The mechanisms by which fibrous tissue influences neo-osteogenesis in a defect or wound area are not completely understood. Ogiso et al. 1991, have shown that fibroblasts produce factors that inhibit bone cell differentiation and osteogenesis. Schmitz and Hollinger 1990, propose that bony union may depend on the cellular ability to calcify the matrix, requiring appropriate growth and differentiation factors in large bony defects.

Guided tissue regeneration is brought about by the differing proliferative rate of tissue types. Fibrous tissue is able to encapsulate the implant quickly, thus preventing osteogenic cells from using the vital dead space they require to propagate. Isolating an area into which fibrous tissue cannot proliferate allows bone to form at a greater volume and reduced porosity. The membrane functions by preventing the haematoma from being invaded by non-osteogenic cells, thus maintaining an osteogenic environment in the defect. However, these experiments do not claim that a bone compartment can be isolated exclusively by e-PTFE, since other materials have also been used to this end (Piatelli et al. 1996).
The lack of any adverse reaction from the soft tissue or bone to the glue should be noted. It is not anticipated that this will provide the main, long-term method of membrane fixation. The glue acts only as an initial stabiliser, holding the membrane in the correct position. Long-term location is maintained by the tissues integrating themselves throughout the membrane structure.

Although commonplace in periodontology, and tested in a variety of interfaces (Béllon et al. 1996), this membrane has yet to fulfill its potential in orthopaedics.

The enhancement of bone regeneration by preventing fibrous tissue ingression has been demonstrated in a single femoral defect, as well as across a defect surrounding a transcortical titanium screw. It is not our intention that these membranes be removed, since their presence physically seals off the interfaces. In theory, a secondary seal may also be produced by the osteopromotive effects of the e-PTFE membrane, enhancing bone formation around the implant.
Chapter 4 Tribology and The Need for Wear Testing

4.1 Introduction to Basic Tribology
4.2 Wear Mechanisms
4.3 Lubrication Types
4.4 Measurement of Surface Roughness and Topography
4.5 Human Hip Joint Tribology
4.6 Arthroplasty Tribology
4.7 Introduction to Wear Testing
4.8 Materials and Methods
4.9 Results
4.10 Discussion
4.11 Summary & Conclusions

4.1 INTRODUCTION TO BASIC TRIBOLOGY

Tribology is defined as 'the science and technology of interacting surfaces in relative motion.' Its application to orthopaedics is clear when the effect of particulate material is understood. Wear will occur whenever surfaces move over each other, generally involving progressive redistribution of material. Early work in tribology focussed in the area of friction, since frictional effects are more readily demonstrated and measured. Friction is defined as 'the tangential resistance, which is offered to the sliding of one body over another' (Hutchings 1992).

The overall aim is to prevent the loosening that occurs in hip joints after approximately the first decade of their lifetime. Experimental testing for this period would be inappropriate in this context. Hence, the aim was to define a surface roughness that would produce an accelerated number of wear particles of the correct morphology with the potential to cause loosening. The definition of this surface morphology forms the basis for the work presented in this chapter.
4.2 WEAR MECHANISMS
For systems consisting of common materials, such as metals, polymers, and ceramics, there are a number of mechanisms by which wear and surface damage can occur (Hutchings 1992). This leads to the generation of wear particles.

4.2.1 Adhesive Wear
This is also termed ‘sliding wear’, and occurs when solids in contact, adhere. When two solid materials are in contact under load, intermolecular attractions are considerable, especially in small areas of contact. If the junction formed is weaker than either solid, interface shear will occur and the surfaces will slide. If the junction is stronger, shear will occur not at the interfaces, but at some distance within the weaker material. This manifests itself in moving articulations of surfaces that are similar materials, or with slow, small surface areas. Hence the adhesion concept suggests that dissimilarity should be sought in choosing pairs of solids which will be in tribological contact. Thus ceramic articulated with polyethylene is a possible method for reducing wear. Continuous sliding leads to the formation and destruction of individual asperity contacts. Adhesive wear is associated with the transfer of fragments of material from the asperities of the polymer to the hard metal surface.

For many systems, the loss of material by wear is proportional to the sliding distance. An initial ‘running-in’ period is sometimes observed at the start of sliding until equilibrium surface conditions have been reached. Scuffing, scoring and gallling describe local solid state welding between sliding surfaces, as well as implying scratching by abrasive particles.
4.2.2 Abrasive wear

Abrasive wear removes or displaces material by microcutting involving a hard particle or shape which indents, grooves, and then cuts material from the surface. A distinction is often made between two body and three body wear, as demonstrated in figure 4.1.

Two-Body Wear Three Body Wear

Figure 4.1 Different Abrasive Wear Mechanisms

Third bodies in arthroplasty include cement, bone, metal, polyethylene and ceramic particles. These wear mechanisms are especially significant in the degeneration of arthroplasty components.

Wear rates are highly influenced by the particle material, size and shape. In engineering applications angular particles cause greater wear than rounded ones, and smaller particles cause proportionately less wear than larger particles (Hutchings 1992).

Wear of the polyethylene due to surface scratches on the femoral head is one of the chief mechanisms of wear. During manufacture, the surface finish of the femoral heads is strictly controlled.

4.2.3 Chemical/Corrosive Wear

These involve wear processes in which chemical or electrochemical reactions with the surrounding environment predominate. This includes the formation of oxide layers over common metals, the properties of which depend on the adherence of the layer. A dichotomy exists between the pacifying effect of the oxide layer and the increased susceptibility of damage to a metal covered in the oxide layer.
Perpetual shear stresses will remove the oxide layer, exposing the underlying metal to the surrounding environment. A new oxide film forms in seconds but over a longer period of time the congruency of the metal will be altered. The oxide layer may also have asperities, damaging the bearing surface.

4.2.4 Fretting wear and Fretting Corrosion
This is defined as oscillatory movements of 1 to 100\(\mu\)m between two solid surfaces in contact (Hutchings 1992). This is especially relevant in modular hip systems (Lombardi et al. 1989, Fricker and Shivanth 1990, Cook et al. 1994). The most significant problems associated with fretting wear are the release of metal ions and particulate debris, seizure of articulating components (more relevant in hinged devices), and galvanic and crevice corrosion. Fretting corrosion occurs when the debris is generated as a product of a chemical reaction between constituents of the surface and the environment, under oscillatory conditions.

4.2.5 Corrosion
In vivo galvanic and crevice corrosion occurs in metals. If two metals with different electrochemical reactivities can conduct, one becomes anodic with respect to the other and corrodes more quickly. This is galvanic corrosion. Crevice corrosion occurs if variations in the metal surface make particular areas inaccessible and have a reduced local oxygen concentration. Corrosion has been thought to occur in modular femoral components (Collier et al. 1992, Cook et al. 1994). Corrosive principles are also considered relevant for metallic particulate matter in vivo (Shahgaldi et al. 1995).
4.3 LUBRICATION TYPES

Lubricants are used to reduce the frictional forces between surfaces. There are a number of different lubrication mechanisms described in order of decreasing lubricant film thickness with the thickest film first.

4.3.1 Hydrodynamic lubrication
Surfaces are separated by a thick fluid film relative to the height of the asperities. The hydrostatic pressure in the film causes small distortions of the surfaces. This type of lubrication is aided if opposing surfaces are geometrically conforming, separated by a small gap over a relatively large area. Squeeze film lubrication can occur when surfaces approach one another.

4.3.2 Elastohydrodynamic lubrication
A thin film under very high pressure will cause elastic deformation of the surfaces. Soft elastohydrodynamic lubrication occurs with a soft surface. It is in this phase that the transition from hydrodynamic to boundary lubrication begins, as mixed lubrication. Synovial joints undergo microelastohydrodynamic lubrication (Dowson et al. 1986).

4.3.3 Boundary Lubrication
Under very high contact pressures, or at lower sliding speeds, direct contact occurs between the asperities. Surfaces are separated by adsorbed molecular films, usually laid down by the lubricating surface with appreciable junction formation and asperity contact. The surface properties of the solid and the lubricant's chemical nature, rather than viscosity, determine the wear between the two surfaces.

4.3.4 Solid lubrication
Solid materials, which exhibit low coefficients of friction, may be used as lubricants in preference to liquid or gas films. Solid lubricants function by providing a solid interfacial film of low shear strength.
4.4 MEASUREMENT OF SURFACE TOPOGRAPHY

A common method of studying surfaces is by use of a stylus profilometer. While the stylus moves over the surface, the vertical displacement produces an electrical current. This signal is amplified and can either move a pen across a chart-recorder or display an average of the displacements over a given length along the surface. A schematic is shown in figure 4.2.

![Schematic diagram of a simple stylus profilometer](image)

A schematic diagram to show the principles of operation of a simple stylus profilometer (Thomas TR 1982)

Figure 4.2 Profilometer Schematic

The trace produced by a profilometer presents surface slopes that are much steeper than they really are, due to distortions in horizontal compression. All stylus methods also produce smoothing of the profile due to the thickness of the stylus tip.

The measure quoted for surface roughness is the average roughness (symbols: $R_a$, c.l.a. for 'centre line average', or AA for 'arithmetic average'). $R_a$ is defined as the arithmetic mean deviation of the surface height from the mean line through the profile, as illustrated in figure 4.3. The mean line is defined so that equal areas of profile lie above and below it (Hutchings 1992).
Mathematically the average roughness, $R_a$ is defined by:

$$R_a = \frac{1}{L} \int_0^L \sqrt{y(x)} \, dx$$

Where $y$ is the height of the surface above the mean line at distance $x$ from the origin, and $L$ is the length of the profile under examination. $R_a$ does however give no indication of the shapes or spacings of the surface irregularities.

4.5 HIP JOINT TRIBOLOGY
The natural synovial joint is lubricated by a synovial fluid film, separating the articular cartilage surfaces. The surfaces consist of water (c. 75%), enmeshed in a network of collagen fibres and high molecular weight proteoglycans. This reduces the coefficient of friction by a combination of elastohydrodynamic, boundary and squeeze film lubrication. During the swing phase, the lubrication is hydrodynamic (Dowson et al. 1986, Black 1992). During stance, heel strike, weight bearing, or toe-off other lubrication mechanisms such as squeeze film, microelastohydrodynamic and boundary lubrication operate. It is beyond the scope of this work to give a detailed description of the tribology of the natural synovial joint. It has been characterised in the literature (Sokoloff 1978, Freeman 1979, Stockwell 1979, Unsworth 1991).
4.6 ARTHROPLASTY TRIBOLOGY

There is a high variability in both the magnitude and type of stresses that arthroplasty components are exposed to. In the replaced hip, wear occurs at both the femoral and acetabular components.

4.6.1 Wear of metallic components

As described in the introduction to tribology, the type of wear mechanism that takes place depends on the lubrication, material, stresses, motion, and surface roughness of the material. For femoral components of the same material, polyethylene wear can be greatly increased with a rougher head (Weightman and Light 1986, Dowson et al. 1987, Fisher et al. 1995, Kusaba and Kuroki 1997). It was this concept that we used to artificially increase wear rates.

Most commonly used surgical alloys have a 2-5nm thick, optically transparent, passive oxide layer. It forms very quickly upon exposure of chromium and titanium to oxygen in the atmosphere or in the body (McGovern et al. 1996). Abrasive wear interfering with the articulation can be reduced by increasing the surface hardness of the materials concerned (Davidson et al. 1994). The opposite view to help solve the wear problem has been postulated (Unsworth et al. 1981) with the use of softer, elastomeric surface materials. Frictional concepts have been reviewed recently in the literature (Hall et al. 1997).

4.6.2 Wear of polymeric components

It is thought that the majority of the wear volume (not wear particle number) in metal/ceramic on polyethylene articulation is polymeric (Shanbhag et al. 1994). Subsequent cellular responses are dominated by the effect of particulate polyethylene (Harris 1994). Hence an understanding of its production is critical in wear prevention, and a brief summary is given below.
The mechanisms for polyethylene wear debris production have been reviewed using surface roughness as an index. Three main wear processes are described (Fisher 1994).

Wear Process (1) produces polymer particles as a result of fatigue failure within the surface of the polyethylene, caused by the cyclic action of microscopic asperities on the femoral counterfaces with an $R_a$ below 0.03μm. As the asperity height of the femoral component increases this process could be said to form a subgroup, where the polyethylene is removed by micro-cutting by the femoral asperity tips.

Wear process (2) assumes that the effect of the femoral counterface is smooth, and that the polyethylene particles are being produced from asperities of between 1-10μm in height on the polymer surface which deforms elastically, then plastically. These local stress concentrations produce crack propagation within 10μm of the surface. Process (1) and (2) are surface wear phenomena.

Wear process (3) is described as a structural failure, involving the detachment of large sections of polyethylene from high subsurface stresses and is described as delamination in knees (Blunn et al. 1991 & 1992, Collier et al. 1991). Its role in hips is not as significant due to the increased congruency of the joint (Bartel et al. 1986, Wright and Bartel 1986) and reduced stresses.

Yield stress of UHMWPE is approximately 24Mpa (PolyHi 1996). Most of the deformation occurs during contact and sliding takes place in the polymer. The surface finish of the hard counterface strongly influences the mechanism of the resulting wear. When the counterface is smooth, adhesive deformation occurs only superficially. This is termed interfacial wear. A rough counterface will have asperities that cause deeper deformation beyond the polymer surface.
4.7 INTRODUCTION TO WEAR TESTING

The problem of wear and aseptic loosening has been discussed in chapter 1. It would be difficult to study this multi-faceted system by *in vitro* methods only.

The development of an animal model, which replicates loosening of a total hip replacement, is a necessary component of studying the biology of wear induced osteolysis, with a view to subsequent prevention.

It is proposed to produce an animal model that accelerates the wear process by using roughened femoral heads. However, in order to produce an accurate *in vivo* model, there must be a certain amount of *in vitro* testing to clarify the relevant parameters involved.

There have been a number of *in vivo* studies that have attempted to replicate the wear/implant/bone interactions that result in particulate induced osteolysis (Howie *et al.* 1988, Goodman *et al.* 1990 and 1995, Bobyn *et al.* 1995, Kraemer *et al.* 1995, Allen *et al.* 1996, Sacoman *et al.* 1996, Kobayashi *et al.* 1997, Hasselman *et al.* 1997, Shanbhag *et al.* 1997). These studies have relied on the injection of wear particles, as a bolus, into the joint space or around the femoral stem.

For example, an uncemented canine THR osteolytic model, which incorporates a bolus of 1 billion polyethylene particles during the operative procedure, has been developed (Shanbhag *et al.* 1997). This cementless model does not attempt to address the implications associated with the continual release of particles from a joint.
The model of Shanbhag et al. (1997) was dependent on a number of factors, including the use of a prosthesis that is specifically designed to facilitate the particulate effect. It cannot be applied to the testing of other prosthetic designs, such as cemented femoral stems. Since the model introduces wear particles at the bone-implant interface, it negates any effect of sealing the bone-implant interface with cement, porous surfaces or any other means.

A small animal model has been developed, with continuous infusion of polyethylene particles (Kim et al. 1998).

A large animal femoral loosening model that attempted to release steady-state particles from the joint has been developed (Vidovsky et al. 1996). However the average roughness of the heads was not given, and so it was not possible to reproduce the model. In this study there was no data given, regarding the particle size, shape and number, as well as wear volume and factor data.

To develop a goat animal model which simulated osteolysis in humans more accurately, it was proposed that precise roughening of the femoral head would lead to quantifiable wear. It was hypothesised that a significantly larger number of particles than normal would be generated by this method. Thus it would be possible to replicate the loosening of hip replacements after 5-10 years duration, at an accelerated rate, in such an experimental model. To select the appropriate surface roughness needed to generate a realistic number of particles of the correct size and shape, this in vitro test was performed.

The effect of individual scratches has been examined (Fisher *et al.* 1995), but this was not intended to be representative of the actual morphology noted in femoral head retrievals.

Particles produced from two different counterface roughnesses (Hailey *et al.* 1996) have been compared with wear particles isolated from around THRs, but no indication was given of surface morphology or how the surface profiles were actually generated.

The objective was to define a reproducible surface morphology and roughness that would yield this accelerated wear rate, with particles of a similar size and shape to those found in human loose total hip replacements.
4.8. MATERIALS AND METHODS

(a) Generation of surface roughness

To test the use of grit paper as a viable method for achieving a consistent surface roughness ($R_a$) over the whole surface of a pin, 5 trial CoCr pins were roughened on 1200, 600, 400, 240, and 120 grit paper (Buehler-Krautkramer, Coventry). The number is inversely related to the size of the silicon carbide particle impregnated onto the paper, and hence the roughness produced. This grit paper is routinely used in this Centre for grinding down undecalcified bone sections containing metallic and/or polymeric implants, embedded in methacrylate resin.

$R_a(\mu m)$ is the universally recognised, and most widely used, international parameter for surface roughness. It is limited since very different morphologies can have similar $R_a$ values hence other parameters are necessary to accurately define a surface. These include $R_{tm}$, which is the average peak-to-valley distance, and $S_m$, which describes the mean spacing between profile peaks at the mean line. Using surface measuring equipment Talysurf, (Taylor-Hobson, Leicester, UK.) the $R_a$ was assessed across the diameters in two perpendicular planes by taking eight values, 0.08mm in length, with a vertical sensitivity of 0.01 $\mu m$. The surfaces were repolished and re-roughened, and the measurements were repeated twice for each grit value. This ensured we had a reproducible roughening technique that produced $R_a$ values, of little statistical variance. The pins were sputter-coated with gold palladium and observed under 15-25kV in a Jeol Scanning Electron Microscope (JEOL JSM 34C, Mfr. Jeol Ltd., Tokyo, Japan) in order to compare morphologies.

Grits 600, 240, 120, and polished controls were selected for the first experiment. The actual test pins were roughened by hand in the same method as the prototypes, using a random motion over the various grit paper roughnesses.
(b) Wear test Configuration
We used a twelve-station pin-on-plate wear test configuration, where a plastic plate was reciprocated against a loaded, rotating CoCr cylinder (Figure 4.4).

Figure 4.4 Test Rig for Abrasive Wear Test

Twelve cylinders of cast CoCr molybdenum alloy, with a spherical end radius of 20mm, were ground, lapped and finished using polishing mops. Pre-test average roughnesses ($R_a$), mean peak-to-valley distances ($R_{tm}=R_z$), and inter-peak spacing distance readings ($S_m$) were recorded over the surface of the pins. Unfortunately $R_a$ was the only parameter recorded during, and after the test had been completed, since the equipment for measuring for $S_m$ and $R_{tm}$ measurement was not available. Each CoCr pin was articulated against GUR 415 Compression moulded Ultra High Molecular Weight Polyethylene (UHMWPE) supplied by Poly Hi Europe. The discs had been sterilised in air by irradiation at 25kiloGrays (2.5 Mrads) 1 week prior to testing.
(c) Test Protocol

The sliding displacement was 5mm at 1Hz, with an in phase synchronised rotational component of 5\(^\circ\) from the midpoint. The average mass of goats used in the subsequent animal study was 60kg. It has been shown that in sheep the maximal force through the hip joint is 110% body weight (Bergmann et al. 1984). Given the available data the calculated force through the goat hip replacement will be 0.66kN. We have chosen a constant force of 0.65kN per indentor. A schematic of the kinematics of the test is shown in figure 4.5.

![Figure 4.5 Test Schematic](image)

The articulation was lubricated with 25% newborn calf serum (JRH Biosciences. HARLAN Sera-Lab, Loughborough) and the test was run for 1 million sliding cycles at room temperature, equivalent to 10km.

Conservative estimates of human activity are noted as 1.8 million steps per year by Seedhom and Wallbridge 1985. This does not correspond to the average amount of displacement seen at the articulation.
(d) Specimen characterisation

Every 2.5km the test was stopped and both the pins and polyethylene discs were removed from their brackets. They were washed with distilled water and placed in an oven at 37°C for 24 hours to allow recovery of deformation and loss of imbibed fluid. The pins and discs were weighed to an accuracy of 0.1mg. Each of the CoCr articulating surfaces had $R_a$ readings measured by taking seventeen 0.08mm average roughness readings at every other point, taken at two repeatable perpendicular planes across the radii of the pins. Unfortunately, $R_m$ and $S_m$ could not be measured during or after the test. Weight losses from polyethylene test samples were compared against the weight change in the control polyethylene discs of the same dimensions soaked in 25% newborn calf serum. Following the analysis the apparatus was re-assembled and continued every 2.5km.

After 10km, the discs and CoCr cylinders were weighed and re-profiled as described. The femoral indentors were soaked in water with 10% Decon 90 for 24 hrs to remove surface contamination, and examined under the SEM as described.

The test was repeated, but the surface finish and morphology of the cylinders was adjusted, by removing the tips of the surface asperities. Hypothetically, partially polishing the surface of the pins would remove the sharper ‘spikes,’ allowing the grooves in the troughs to produce smaller polyethylene particulate debris by abrasive means between the peaks, rather than sharp projections gouging out large masses of polyethylene.

A shot-blasted surface and grit paper 1200 roughened surface was tested, as well as partially polished surfaces roughened by grit papers 1200, and 600.
(e) Digestion of serum and Particulate inspection

Acid digestion (Margeviucius et al. 1994) of the serum consistently produced well separated particles for examination and quantification under the SEM. 0.25ml of wear particle-containing serum had 1ml of filtered 10M HNO₃ added to it. This was left for 24hrs at 60°C in an electrically heated oven. Prior to filtering the fluid was diluted with filtered distilled water to 40ml. The polyethylene-containing serum was passed through a 0.2μm filter (Nucleopore® Polycarbonate. G3831 Agar scientific, Stansted) which was washed with an additional 20ml of distilled water, and 10ml of air to prevent loss of the polyethylene suspension when the filter was disassembled. The filter was dried in an oven at 37°C for 24hrs, and prepared for sputter coating as described.

To quantify the average particle diameter, three representative scans were taken for each of the three serum samples per pin roughness. The magnification for particle quantification was X1000. Particles were allocated to maximal diameter groups, in the regions of 0.2-0.9, 1-4.9, 5-9.9, 10-49.9, 50-99.9, and 100-500μm. Approximately 200 particles were counted in each scan.
4.9 RESULTS
(a) Generation and Characterisation of Surface Roughness
The reproducibility of $R_a$ values, using grit paper techniques of different roughnesses, was confirmed by the low standard error values as shown in figure 4.6.

![Figure 4.6](image)

Figure 4.6 Rougher papers produce a higher average Roughness
Reproducibly generating the $R_a$ was achieved by abrading the surface over an unused area of the grit paper, forming definite scratches (Bousfield 1992), as noted in the SEM images of Figure 4.7 and 4.8 (Mag. x100, x1500, $R_a$ 0.3μm), scratched with 400 grit paper.

![Figure 4.7](image)

Figure 4.7 Scratched CoCr Surface (X100)
These results can be compared to those from retrieved femoral heads (Fisher et al. 1994, Jasty et al. 1994, McGovern et al. 1996), indicating that grit-roughened surfaces correspond more closely to retrieved femoral heads than shot-blasted surfaces, as shown in figure 4.9.
Moreover, retrieved cups exhibit a similar wear morphology to the grit roughened surfaces (Jasty et al. 1997), scratched by the same mechanisms.

The $R_a$ values for each of the CoCr cylinders labelled 1 to 12, for the first experiment, are shown in table 4.1.

<table>
<thead>
<tr>
<th>Pin number</th>
<th>Roughening used</th>
<th>Mean tip $R_a$</th>
<th>St. Dev.</th>
<th>St. Err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>None. Polished controls</td>
<td>0.040</td>
<td>0.036</td>
<td>0.005</td>
</tr>
<tr>
<td>4-6</td>
<td>600</td>
<td>0.177</td>
<td>0.035</td>
<td>0.005</td>
</tr>
<tr>
<td>7-9</td>
<td>240</td>
<td>0.377</td>
<td>0.058</td>
<td>0.008</td>
</tr>
<tr>
<td>10-12</td>
<td>120</td>
<td>0.845</td>
<td>0.177</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Table 4.1. Average roughness values of pins for the first Test

Figures 4.10 and 4.11 show the mean peak-to-valley distances ($R_{tm}$) and the mean spacing between profile peaks at the mean line ($S_m$):

Figure 4.10 Rougher surfaces Increase the Height of the Asperities
Following examination of the SEM micrographs and the particulate debris, we hypothesised that the grit paper created sharp edges on the CoCr pins that would cut into the polyethylene and cause removal of larger bulk material. We had anticipated that the grooves in the metal would produce particles of a consistent form, facilitated by the removal of the sharp tips from the surface asperities.
Chapter 4

The $R_a$ values for the repeated test are shown in table 4.2. The two readings taken across each pin were not statistically different from one another in any of the test specimens.

<table>
<thead>
<tr>
<th>Pin number</th>
<th>Roughening used</th>
<th>Mean tip $R_a$</th>
<th>St. Dev.</th>
<th>St. Err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-15</td>
<td>Shot-blasted</td>
<td>0.258</td>
<td>0.055</td>
<td>0.007</td>
</tr>
<tr>
<td>16-18</td>
<td>Polished 600</td>
<td>0.138</td>
<td>0.045</td>
<td>0.006</td>
</tr>
<tr>
<td>19-21</td>
<td>Normal 1200</td>
<td>0.137</td>
<td>0.034</td>
<td>0.005</td>
</tr>
<tr>
<td>22-24</td>
<td>Polished 1200</td>
<td>0.080</td>
<td>0.035</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 4.2 Average roughness values of pins in second test

(b) Deformation of the wear track

The rate of volume loss is shown by Figure 4.12. In all graphs the vertical bars represent standard errors of the mean.

It should be noted that these results are the profile obtained from the polyethylene discs, and represent both deformation and wear. A large difference in volume loss was noted between the rougher and smoother pins. Rougher pins also produced 'rippling' in the floor of the wear track.
The change in volume was greatest after the first 2.5km, indicating that there was a substantial plastic deformation of the polyethylene due to a low initial contact area resulting in high pressure. The volume changes for the discs roughened at 120 and 240 were not statistically different from one another. The use of polishing to remove the surface spikes from pins roughened with papers 600 and 1200 produced volume changes in the discs that were neither statistically different from each other, nor from the normal 600 and 1200 roughening. The absence of any polyethylene build-up around the sides of the wear track showed that the majority of the deformation occurred in the bulk material beneath the track. A clearer representation of the volume loss for the smoother surfaces is shown in Figure 4.13. For reference, the dotted lines represent wear volumes between 10 and 100 mm$^3$ per year, as reported in the literature (Wroblewski 1986, Livermore et al. 1990, Fisher and Dowson 1991). The penetration of the indentors also fell within reported rates of femoral heads in Charnley acetabular sockets of 0.07-0.22mm/year (Atkinson et al. 1985), for all of the roughnesses.

![Figure 4.13 Volume Loss from polyethylene discs(2)](image-url)
Figure 4.14 shows the average mass changes of the discs throughout the test, with smoother pins shown more clearly in Figure 4.15.

![Graph showing weight loss from polyethylene discs](image1)

**Sliding Distance (km)**
Figure 4.14 Weight Loss from polyethylene discs(1)

Polished control discs seemingly gained weight at 10 km. This is thought to be due to inaccuracies of as a result of soak control discs that were unloaded.

![Graph showing weight change from polyethylene discs](image2)

**Sliding distance (km)**
Figure 4.15 Weight Loss from polyethylene discs(2)
Figure 4.16 shows the wear factor as calculated by dividing the wear volume by the load multiplied by the displacement, plotted against the grit paper used for roughening the pins that were not subsequently polished. It demonstrates that the wear factor is related to the $R_a$, and is shown to be linear for grit papers 600 to 120.

![Wear factor graph]

**Figure 4.16 Wear factor expressed for each grit paper**

(c) Changes in the femoral CoCr indentor.
Macroscopic examination of the femoral components for grits 600, 1200, and polished 600, 1200, revealed obvious wear effects on the pin surface. A circular, more polished area could be seen at the pin tip. Under the SEM a difference was observed between the surfaces which had been roughened then polished, and those just roughened, confirming the removal of the "cutting spikes." Many of the femoral indentors had evidence of polyethylene adherent to the surface, as in Figure 4.17. The polished asperities can also be seen.
Figure 4.17 SEM Photomicrograph Showing Adherent Poly Particle

Figure 4.18 demonstrates that only the polished 600 and the 1200 grit roughened pins polished at 10km of sliding.

Figure 4.18 Graph demonstrating extent of polishing of the CoCr pins
(d) Particle characterisation

With all pin roughnesses, polyethylene debris was produced. Since the extracting volumes for each serum sample were the same, the particle numbers and size distribution were comparable. $R_a$ values of 0.845 $\mu$m (120 grit) and 0.377 $\mu$m (240 grit) produced a variety of particle sizes and large amounts of polyethylene debris. This was evident from the appearance of the serum samples that were 'cloudy' relative to those of the smoother conterfaces. Figures 4.19, 4.20, and 4.21 show the representative particles obtained from grit papers 240(0.377 $\mu$m), 600(0.177 $\mu$m), & 1200(0.137 $\mu$m) respectively, with $R_a$ values shown in brackets.

![Figure 4.19 Polyethylene Particle from Grit Paper 240](image)

Figure 4.19 shows one larger particle, surrounded by a number of smaller particles of varying sizes. The 600 grit produced both medium sized particles and smaller particles. Figure 4.21 shows a number of smaller particles, with no larger polyethylene material noted, as seen in the previous two figures.
Figure 4.20 Polyethylene particle from grit paper 600

Figure 4.21 Polyethylene particle from grit paper 1200
Two higher mag, images at X6000 are shown of a particle obtained from grit 600 and from grit 240, in Figures 4.22 and 4.23 respectively. It can be seen that the morphology of the polyethylene agglomeration of Figure 4.23, is made of the central region of single particles noted in figure 4.22.

Figure 4.22 Polyethylene particle at a larger Mag. from grit paper 600

Figure 4.23 Polyethylene particle at a larger Mag. from grit paper 240
Particle morphology was dependent on the particle size since large particles (100μm) are dissimilar to smaller particles (1μm). The larger particle morphology, produced only from the rougher 240 and 120 surfaces, has not been seen in human hips. However, it has been noted in other in vitro wear testing experiments (Fisher et al. 1995, Wang et al. 1996).

The morphology of the smaller polyethylene particles from grits 1200, and 600, (X6000 grit 600) has been seen in retrievals (Margeviucius et al. 1994, Shanbhag et al. 1994, Campbell et al. 1995, Baslé et al. 1996, Hirakawa et al. 1996a&b, Wolfarth et al. 1997).

It could be seen at X6000 magnification many of the larger particles resemble "fused" smaller particles, described as similar to 'cauliflowers' (Shanbhag et al. 1994). They had an elongated tail region with a rounded end component (Campbell et al. 1995). In contrast, the majority of particles from the smooth counterfaces are of the rounded morphology.

As the pins became smoother, the number of larger particles decreased and the total number of particles produced was less, as represented by Figure 4.24. The graph in Figure 4.24 also illustrates the distribution of polyethylene particles recorded, represented as a percentage of the total found. Rough counterfaces produced both large and small particles.
Smoother counterfaces produced fewer particles overall, but the percentage of smaller particles was higher. Many of the small particles from all scratched surfaces had the same "head and tail" appearance as noted earlier.
4.10 DISCUSSION

The surface morphology of the pins used in this wear test greatly influenced the wear of UHMWPE. Wear of the shot-blasted surfaces does not appear to be similar to the in vitro wear surfaces of a similar roughness, nor to wear seen on retrieved femoral heads. To define only the surface roughness, without characterising the actual morphology over the metal counterface, is insufficient in describing or predicting wear data. Any work that has described the role of surface roughness on the wear of UHMWPE needs to consider this.

When Jasty et al. (1994) examined retrieved femoral heads they noted, “microdirectional fine scratches which appeared to have been made by fine, hard, particles.” In light of this it is perhaps not surprising that the fine, hard silicon carbide particles impregnated into grit papers produce wear morphologies similar to those of retrieved femoral heads. Consequently to make wear particles of a similar size and shape to those found around loose hip replacements; the mechanisms by which they are produced should be replicated.

The variation in particle size noted in the hip capsule tissue would indicate that there a number of wear mechanisms taking place. These wear processes have been described by co-workers (Fisher and Dowson 1991, Lancaster 1991, Cooper et al. 1993, Fisher 1994, Fisher et al. 1995).

A polished CoCr surface is extremely smooth, with an asperity height in the order of 0.01 μm. The asperities on the polymer, 1-10 μm, deform in an elastic and plastic fashion, producing stress concentrations and fatigue failure of the polyethylene up to 10 μm under the polymer surface. For the smoother counterfaces, asperity wear processes are dictated by adhesive and fatigue mechanisms (Cooper et al. 1993).
The grit paper created surface grooves flanked by surface peaks. As the surface $R_a$ increased the surface peaks abraded and cut into the polyethylene, producing wear particles. The average roughness of 120 grit was $0.79\mu\text{m}$.

If all of the peaks were triangles of the same height, the average roughness would be half the peak to valley value. For an $R_a$ of $0.84\mu\text{m}$ (120 grit), this height would not be large enough to produce a polyethylene particle instantaneously, of up to $500\mu\text{m}$ in diameter if using only abrasive methods. A secondary wear mechanism was therefore responsible. The spacing parameters demonstrate that there is a larger difference in the peaks for the rougher surfaces. Groove width will have initially been determined by the sizes of the individual silicon carbide particles on each of the grit papers. The U.S. average carbide sizes for papers 120, 240, 600, and 1200 are 127, 58, 25, and $15\mu\text{m}$ respectively. If one assumes that the particles create the grooves, and considering that these surfaces are loaded with both a displacement and rotational component, the responsibility of larger particle generation lies with the grooves, or the *inter*-peak distance. The polished surface also had a large inter-peak distance. When the average roughness data is considered in tandem, it is apparent that there is at least an order of magnitude in the peak-height, but the distances are similar, as expected in a polished surface.

The 'rippling' effect on the floor of the wear tracks for pins 120, and 240, with the highest $R_a$ values of $0.845\mu\text{m}$ and $0.377\mu\text{m}$, was thought to be the initial delamination of the polyethylene surface. This was brought about by the higher coefficient of friction for the rougher surfaces, and the subsequent development of high sub-surface shear stresses (Suh 1986).
As noted in the results, the change in deformation was greatest after the first 2.5km. Much of the volume loss would not be due to the liberation of particulate debris, but to plastic deformation. True volume loss was calculated by dividing the mass of the polyethylene by the density, \( \rho \).

When the maximum shear stress of the UHMWPE reached the shear strength of the material, plastic deformation was inevitable. As the test continued, the deformation of polyethylene decreased, due to the increase in contact area, thereby reducing the stress. Much of the subsequent loss of volume was then due to polyethylene wear on the abrasive pin surface. For the computation of the wear factors, the calculated volume values were used.

Counting the particles, and dividing them into particle size groups was a potential drawback in overall particle assessment. The SEM images gave little indication whether a large particle, is indeed a large particle by itself, or an accumulation of smaller particles, with indistinguishable margins.

A wear model is designed to replicate the conditions that exist in vivo. This is corroborated by the extremes of two experimental criteria; either the motion and forces of the human condition are duplicated by a machine, or data obtained in vivo is replicated by an in vitro model. In the construction of an in vivo model, ideally the in vitro set-up would have facilitated a hip simulator, compensating for the variation in geometry and kinematics. Femoral head surface retrievals have affected areas and unaffected areas with marked demarcation between zones (Jasty et al. 1997). This indicates that the load distribution and motion over the conforming bearing surface is not evenly distributed; this may also explain the diverse particle sizes found in retrievals.
4.11 SUMMARY & CONCLUSIONS

This wear test has described a methodology for the steady-state production of wear debris in order to establish an experimental wear debris induced osteolytic model. It also demonstrated the role of surface roughness and surface morphology in debris production from the CoCr counterfaces.

An intermediate grit between 1200 and 600 was selected to roughen the goat femoral heads; grit 800. This was correlated to the wear factors and rates and deemed the most appropriate for the purposes of the goat experimentation as detailed in the next chapter.
Chapter 5 The Prevention of Wear Debris induced Osteolysis Using Guided Bone Regeneration

5.1 Introduction

5.2 Materials and Methods
   5.2.1 Femoral Stem Manufacture
   5.2.2 Acetabular Cup Manufacture
   5.2.3 e-PTFE Dimensions
   5.2.4 Insertion of Prosthetic Components
   5.2.5 Post-Operative Regime: Short Term
   5.2.6 Post-Operative Regime: Long Term
   5.2.7 Implant Retrieval
   5.2.8 Tissue Processing
   5.2.9 Basic Statistics

5.3 Results
   5.3.1 Femoral Head Data
   5.3.2 Head Penetration Data
   5.3.3 Tissue Digestion Data
   5.3.4 Radiographic Evidence
   5.3.5 Bone Histology
   5.3.6 Soft Tissue Histology

5.4 Discussion
   5.4.1 Femoral Head Data
   5.4.2 Acetabular Cup Wear and Femora Head Penetration
   5.4.3 Radiographic Evidence
   5.4.4 Bone Histology
   5.4.5 Soft Tissue Histology and Digestion Data

5.5 Summary and Conclusions
5.1 INTRODUCTION

The previous chapters described the investigations into the biocompatibility and the bone regenerative capacity of e-PTFE used in conjunction with butyl-cyanoacrylate glue. These biomaterials are proposed for use in the prevention of osteolysis in a goat model with an intention to use these materials in the human context. The aim was to seal the interfaces from the joint space by physical seal, that would become biologically reinforced. This would prevent migration of debris to the joint space.

Maxillofacial and periodontal experience demonstrates that membrane placement, with the exclusion of fibrous ingrowth, enhances osteogenesis. This will form the basis for the secondary biological seal. Hence this membrane could prevent wear particles generated at the acetabular cup-femoral head articulation from migrating along the implant interface, preclude wear particle induced osteolysis, followed by implant loosening. In order to establish whether loosening could be prevented, it was a prerequisite to have a model in which loosening occurs. The previous chapter described the characterisation of a surface roughness which would produce wear particles of a similar morphology and size to those seen in the capsules around loose total hip prostheses after ten or more years. By using femoral heads of this roughness, the number of wear particles produced would be sufficient to reproduce the loosening around hip replacements as presented in humans after five to ten years.

Loosening is a multifactorial process, and procedures used to investigate loosening and methods to compare wear particle induced osteolysis need to be tested in an osteolytic model. One of the objectives was to produce a realistic, one year loosening model by accelerating the wear processes that produce wear debris of appropriate size and numbers. Using this model, the effectiveness of the seal could be tested.
5.2 MATERIALS AND METHODS

5.2.1 Femoral Stem manufacture

The Centre for Biomedical Engineering has a CAD-CAM workstation for the design and manufacture of custom joints and massive endoprosthetic replacements. From examining posterioanterior and lateromedial views of pre-operative goat radiographs, as well as with previous goat hip arthroplastic experience, the femoral stems were manufactured to an “off-the-shelf” size. The stems were constructed from Hot Rolled Annealed titanium Ti6Al4V alloy (Timet, Birmingham, UK). They were machined with a spigot designed to fit Biomet Type 1 tapers. The cemented, tapered stems were of a generic design, with a collar, cement grooves, and modular heads. CoCr heads were purchased from Biomet (Swindon, UK), with an external diameter of 17.9mm (±0.4mm tolerance).

Goats were randomly selected to receive accelerated wear particle producing heads. The CoCr surfaces of the heads were scratched with 800-grit paper (Buehler-Krautkramer, Warwick, UK). The heads had pre-operative $R_a$, $R_{tm}$ and $S_m$ readings taken from the anterior, superior, inferior and posterior aspects of the head surface.

5.2.2 Acetabular Cup Manufacture.

Hoechst GUR4150HP Powder was used to manufacture Tivar® Ram Extruded Rods of moulded Ultra High Molecular Weight Polyethylene (UHMWPE) supplied by Poly Hi Europe. From the rods, the acetabular cups were machined within the Department. They were manufactured with a bore diameter of 18mm. Once machined, the cups were ultrasonically cleaned and sent to Isotron (Reading, UK), for sterilisation by $\gamma$-irradiation at 25kiloGrays (2.5 Mrads).
5.2.3 e-PTFE dimensions

Just as the cups and femoral stem components were manufactured to a pre-determined size, so were the e-PTFE membranes. These dimensions were ascertained by shaping various membrane sizes around cadaveric goat bones, and selecting the most appropriate measurements as represented by figure 5.1 (Numbers in mm.).

![Diagram of Acetabular and Femoral e-PTFE membranes]

Acetabular

Femoral

Figure 5.1 Dimensions of e-PTFE membranes used for implantation

These were cut at W.L. Gore & associates by laser and then packed for steam sterilisation.

The acetabular e-PTFE membrane was symmetrical since the anatomy allows an even spread around the margins of the cup. The femoral membrane was not. The shorter hole-membrane edge distance was placed medially. The medial area of the neck/shaft did not have a wide margin for membrane attachment due to the location of the lesser trochanter, with the con-joint insertion of iliopsoas. Consequently the shorter membrane makes attachment easier onto the bone surface, without interfering with the tendinous insertion.
Chapter 5

The hole in the middle of each of the membranes allows them to be congruently fitted around their respective implants. For the cup, this was fitted prior to cementing and post-cementation for the femoral stem.

5.2.4 Insertion of prosthetic components

The operative procedures were performed at the Biological Services Unit of the Royal Veterinary College (North Mimms, UK). Appropriate animal, project, and personal licenses had been home office approved in accordance with the Animals (Scientific Procedures) Act of 1986. The procedures had also been passed by the ethical committee of the Royal Veterinary College.

The hip joint was accessed by the posterior approach. A comprehensive anaesthetic and surgical description required for total hip prosthetic component insertion has been described in Appendix A1. All operations were carried-out under aseptic conditions.

5.2.4.1 Prosthetic insertion: Controls

There were two groups of animals: controls and test. Each individual animal was clearly numbered with an ear-tag, and neck chain. Eight Control goats had a routine total hip arthroplasty with a total capsulectomy and removal of the soft tissue from around the intertrochanteric region and inferior margins of the femoral neck. The regions around the acetabular cup also had the soft tissue removed 15mm from the margins of the acetabulum. This was a sham procedure in the controls, intended to replicate the soft tissue removal needed to affix the e-PTFE membranes for the test groups, as described below. Figure 5.2 illustrates the procedure in the control group.

106
Two animals in this control group had a smooth, polished head inserted, as opposed to the roughened heads.

5.2.4.2 Membrane placement for Test Groups
Test groups were implanted with the prototype e-PTFE membrane. As with the controls, a periosteal elevator was used to remove any soft tissue on the bone surfaces where the membrane was intended for attachment. It was a critical part of the procedure to ensure genuine bone-membrane contact without the interposition of soft-tissues. A bone bed, onto which the e-PTFE could be glued, had been isolated. This added approximately 15 minutes to the procedure. As mentioned in the introduction to guided bone regeneration, for acceptable results, an intact and stable membrane-bone complex must be created and maintained for an adequate period during healing.
The e-PTFE was attached to the bone using Histoacryl®, butyl-cyanoacrylate (Braun, Melsungen, Germany). The presence of blood caused this adhesive to polymerise therefore it was important to ensure that the bone, where the membrane was to be glued, was as bloodless as possible. As with the controls, two animals in this test group had smooth polished heads inserted.

5.2.4.2.1 Acetabular membrane placement

The UHMWPE cup was cemented. Before cementing, the e-PTFE membrane was stretched to fit over the posterior aspect of the cup, through the hole in the membrane. e-PTFE has a degree of expandability and allows such a procedure to be carried out without tearing the membrane. The e-PTFE was securely located around the cup in the most lateral of the grooves, originally intended for cement macrointerlock. Once this was assembled, the cup was cemented into place, and held in position until the cement had polymerised.

Figure 5.3 Intra-Operative View of Test Hip Insertion
5.2.4.2 Femoral membrane placement
Having inserted the femoral component, the femoral e-PTFE was implanted prior to head impaction. The hole in the membrane was stretch-fit over the spigot and pulled as far down distally as the shoulder and collar of the femoral component would permit. The membrane was then rotated such that the smaller radius was facing medially. In this position, the membrane was glued down to dry bone. In the trochanteric region, it was especially important to maintain membrane-bone contact at the membrane edges. The membrane was also glued to the neck of the femoral stem.

5.2.5 Post-Operative Regime: Short Term
Immediately post-operatively, the goats were permitted to recover, taken back to single pen housing and placed in sternal recumbency to allow regurgitation of any fluids. Goats were allowed to weight bear at will normally. Goats received antibiotic cover with Enrofloxacin (Baytril™, Bayer AG Leverkusen at 3ml/kg) and pain relief using Flunixen (Finadyne™, Schering-Plough Ltd., Welwyn Garden City, U.K at 2ml/45kg) for three days post-operatively.

Project license holders checked the welfare of each animal individually for the next seven days and then once every week.

For the main study, 21 goats received total hip replacements. Originally 16 were scheduled with five extra goats for any unforeseen circumstances, such as post-operative infections, fractures or dislocations, as summarised in table below. Goat numbers are indicated by an R-- number. In all cases the right hip was operated on. The operation dates are in brackets, with all operations performed in 1997.
Table 5.1 Surgical Summary of Goat Operative Procedures

<table>
<thead>
<tr>
<th>Number</th>
<th>Pathology</th>
<th>Test/Control-</th>
<th>Time in Vivo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>R78</td>
<td>Dislocation</td>
<td>Test. Rough.</td>
<td>5mths</td>
</tr>
<tr>
<td>R82</td>
<td>Anaesthetic</td>
<td>Control. Rough</td>
<td>8mths</td>
</tr>
<tr>
<td>R88</td>
<td>Fracture</td>
<td>Test. Rough.</td>
<td>2mths</td>
</tr>
<tr>
<td>R91</td>
<td>Dislocation</td>
<td>Control. Rough.</td>
<td>6mths.</td>
</tr>
<tr>
<td>R93</td>
<td>Infection</td>
<td>Test. Rough.</td>
<td>7mths</td>
</tr>
</tbody>
</table>

5.2.6 Post Operative Procedure: Long Term

The goats were kept within single housing for 4 weeks, and then allowed to graze in the paddock. They were walked for a minimum period each day as detailed in the table below.

<table>
<thead>
<tr>
<th>Time post op</th>
<th>Exercise Regime</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days</td>
<td>4mins exercise daily</td>
</tr>
<tr>
<td>32 days</td>
<td>6mins exercise daily</td>
</tr>
<tr>
<td>34 days</td>
<td>8mins exercise daily</td>
</tr>
<tr>
<td>36 days</td>
<td>10 minutes exercise every day thereafter</td>
</tr>
</tbody>
</table>

Once the hair had grown over the wound site, the goats were allowed to graze normally in the field during the day, and group housed in the evening for overnight protection.
Three sets of radiographs were taken, at 4, 8, and at 12 months post-operatively. This was carried out at the Equine Radiology Department at the Royal Veterinary College. An immediate post-operative radiograph was not taken since this would have required further anaesthesia and hip joint manipulation immediately after surgery.

5.2.7 Implant Retrieval

Animals were culled in two groups. R76, R77, R79, R80, R81, R83, R84, R85 and R86 were culled on the last week of January 1998, and R87, R89, R90, R92, R94, and R95 in the first week of April 1998.

Ten minutes before culling 10,000 units of Heparin was injected IV. The goats were sedated with 20 mg/ml xylazine (Rompun™, Bayer AG Leverkusen), administered intramuscularly (0.005 ml/kg), with 5 mg/ml of Midazolam (Hypnovel™, Roche Products Ltd., Welwyn Garden City, UK) at a dose rate of <1 ml/animal. The animals were culled with 200 mg/ml of Sodium Pentabarbitone (Euthatal®, Rhone Merieux, Essex, UK) via central access at a dose rate of 150 mg/kg.

The animal was placed in the supine position and a “Maltese cross” incision, measuring 50 cm X 50 cm was made over the abdomen centring at the umbilicus. The greater omentum and gut contents were displaced to the left exposing the abdominal aorta. This was verified by palpation of the spinal bodies posteriorly. The aorta and vena cava were dissected and isolated from the surrounding fascia.

At the level of L1/L2 the proximal end of the vena cava and aorta were tied-off. A 12-gauge canula was inserted into the aorta, and a yankee sucker placed into a 5 mm incision made into the vena cava for blood removal. The circulation was flushed with 1.5 litres of saline before perfusion with 1.5 litres of 10% buffered formalin saline. Muscle twitching could be seen as the tissues fixed.
Initially the superficial inguinal lymph nodes were identified. These were removed bilaterally and placed in formalin, for routine paraffin wax histology. A section of spleen was also taken for histology.

The goat was placed in lateral recumbency with the operated right side facing up. The hip joint was removed en bloc, with osteotomies taken at the ischium, ilium, pubis and proximal 2/3rds of the femur.

5.2.8 Tissue processing
There were a number of different tissue types obtained from each of the goats:
1. Resected en bloc hip joint. Some of these were disarticulated after removal from the goat.

2. Joint fluid aspirates were sent for bacteriological assessment and smears were made for investigation of cellular particulate profiles of the joint fluid.

3. From the disarticulated sections, capsule ring biopsies were taken from around the hip joint capsule up to the neck of the femoral component. “Window biopsies,” of small 3mmX3mm squares were taken from the anterior, inferior, superior, and posterior regions of the hip capsule for the joints left articulated.

3. Left and right inguinal lymph nodes

4. Samples of the spleen, measuring 1cmX2cmX3cm.
5.2.8.1 Resected hip joint

The hip joint resection was DEXA scanned upon removal. A radiograph was made of the resected block. The specimen was also photographed and macroscopic examinations were made.

Resection numbers R77, R79, R81, R84 and R86 were left articulated for further histology, whereas the rest of the implants were disarticulated. In four of the rough and four of the smooth samples that were disarticulated, the heads were measured for average roughness (Ra) values as well as average peak-to-valley (Rtm) and inter-peak spacing distances (Sm).

The wear of polyethylene occurs in a particular direction, with the femoral head cutting a cylindrical-shaped path through the material (Charnley and Halley 1975, Dowling et al. 1978, Griffith et al. 1978, Wroblewski 1985). To determine any changes regarding head penetration into the acetabular cups, all of the disarticulated cups had impression moulds taken (Kabo et al. 1993. Hall et al. 1995) with a silicone based condensation curing elastomer (Optosil® P, BayerDental, D-51368 Leverkusen, Germany). These were analysed using shadow-graphing techniques by Dr. James L. Cunningham at the Dept. of Orthopaedics, of the University of Bristol. It was important to measure the tolerances of the femoral heads, for the diameters before and after scratching.

The bones and their associated implants were processed as summarised in the table below:

<table>
<thead>
<tr>
<th>Process</th>
<th>Time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fixed in formalin</td>
<td>10</td>
</tr>
<tr>
<td>2. Saw sections</td>
<td>1</td>
</tr>
<tr>
<td>3. Alcohol</td>
<td>2 changes</td>
</tr>
<tr>
<td>70%.</td>
<td>5</td>
</tr>
<tr>
<td>90%.</td>
<td>6</td>
</tr>
<tr>
<td>100%</td>
<td>9</td>
</tr>
<tr>
<td>4. Resin/Alcohol mix</td>
<td>11</td>
</tr>
<tr>
<td>5. Impregnated with resin under vacuum</td>
<td>21</td>
</tr>
<tr>
<td>6. Cast in resin</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5.3 Processing of en bloc Goat Sections
Having fixed the resections in formalin, they were cut using a slitting wheel, in specific orientations as illustrated by figure 5.4. A coronal section was also taken in the proximal region of the femur and in the acetabulum. These particular sections were only taken after the tissues had been embedded in methacrylate LR Whites resin.

![Diagram of sectioning criteria of goat hip with labels: UNdecalcified, only sectioned after resin embedded, 15mm, 20mm, 15mm, 30mm, calcified, calcified, calcified, calcified, UNdecalcified, UNdecalcified.](image)

**Figure 5.4 Sectioning Criteria of Goat Hip**

The transverse sections were cut after fixation to avoid disruption of the tissues, but before immersion in alcohol to allow a more complete dehydration by facilitating easier access to the volume of tissue. Sections selected for decalcification were placed into the chelating agent EDTA (ethylenediaminetetraacetic acid). They were X-rayed every fourteen days to monitor decalcification. Dense goat cortical bone took up to eight weeks to decalcify.
Once decalcified, the bone slices were dehydrated, cleared in chloroform, double embedded in 1% and 2% celloidin, paraffin wax embedded and sectioned to 5μm on a sledge microtome.

Having divided the sections, the undecalcified specimens were dehydrated in alcohol, and impregnated with Hard Grade London Resin (LR White’s R1230, Agar Scientific, Essex). Resin impregnation was performed under vacuum to prevent the formation of bubbles in the sections.

The specimens were cooled before, during and after polymerisation to reduce the extent of thermal damage to the tissues as the exothermic reaction proceeds. Curing exotherm was important as specimens contained a large amount of conducting metal that could burn surrounding structures.

After embedding, the proximal region of the specimens was sectioned in the coronal plane, as defined in the schematic above. An exotom apparatus (Struers, UK) was used to perform the sectioning.

The resin blocks were trimmed using a band saw in the plane perpendicular to the section for preparation for slide attachment. Technovit® 7210 VLC (Kulzer, Germany) was used as an adhesive to hold the section to the slide whilst the block was brought to approximately 1mm thick on the slide by cutting parallel to the section with a precision diamond saw (Isomet™, Buehler-Krautkramer, Coventry). The undecalcified bone sections were X-rayed at this stage, as described in the next section.
After X-raying, the slides were ground and polished using a dedicated grinder (Motopol™, Buehler-Krautkramer, Coventry). Rougher grit papers (240, 400) were used to bring the sections to approximately 200μm. Then smoother papers (600, 1200) were used to make undecalcified bone/implant sections of approximately 50-70μm in thickness. These were polishing cloths using initially a 5μm lubricant, Alpha micropolish alumina (40-6351-006, Buehler-Krautkramer, Coventry) and then a 0.06μm Colloidal Silica lubricant (95-B1165 Buehler-Krautkramer, Coventry) for final polishing. For some sections, the surface was sputter coated as described and examined by back-scattered SEM.

The sections were washed, and stained with Toluidine blue for 20 minutes, followed by Paragon for 15 minutes. 25ml of Paragon multiple stain was made from 25ml of 30% ethanol, 1.8g Toluidine blue and 0.69g of basic fuchsin (Lalor 1993). Nuclei, cartilage matrix and osteoid stained blue. Mineralised bone stained pink to purple.
5.2.8.3 Radiographic quantification methods

The pre-grind 800μm thick sections were X-rayed (Raymax, Newton Victor Ltd., UK).

5.2.8.3.1 Acetabular Radiographic Quantification

A scale, and a protractor, with 5° delineations marked, was placed over the acetabular cup radiograph. Every 5°, the distances between the cement and bone were assessed in a blinded fashion by an independent reviewer. A scanned image, from a control goat, is given in figure 5.5: This was performed in all of the animal groups.

![Figure 5.8 Scanned Acetabular Section Radiograph](image)

5.2.3.8.2 Proximal Femoral Radiographic Analysis

The cement-bone distance was measured every one mm along the coronal section of the lateral and medial side, by a blinded assessor not connected with the project. Along each of the lateral and medial aspects, 30 and 20 recordings were made respectively.
5.2.8.3.3 Transverse stems.
The amount of radiographic osteolysis in the femora was analysed by blind assessment. The lytic surface area was expressed as a percentage of the total bone area. The cement-bone distance was deemed unreliable for loosening assessment because of the poor initial cement mantles.

5.2.8.3 Joint Fluid Aspirates
The fluid isolated from the joint was cultured in general media separately for aerobic and anaerobic organisms. The joint fluid was also examined under the light microscope under polarised light.

5.2.8.4 Capsule Biopsies
The type of biopsy that was taken from the hip capsule depended whether or not the section had been disarticulated. Resection numbers R77, R79, R81, and R84 were left articulated for histology, whereas the rest of the implants were disarticulated. Hence for the disarticulated sections a complete ‘ring’ of capsule tissue was taken, up to the femoral stem, being careful not to disrupt the gore membrane. These were Haematoxylin and Eosin, Oil Red O, and Trichrome stained for examination and characterisation under the light microscope (Olympus BH2).

Parts of the capsular tissue were digested in order to isolate wear particles for examination under SEM. 20μm ring capsule sections were digested. The wear particle containing hip capsule tissue had 1 ml of 10M HNO₃ added to it. This was left for 48hrs at 60°C and ultrasonicated intermittently for five hours.
The digest was diluted with 20 ml of distilled water and passed through 0.2μm filter (Nucleopore® Polycarbonate, G3831 Agar scientific, Essex). All reagents were pre-filtered using a 0.2μm filter. The filter was dried in an oven at 37°C, for 24hrs. The filters were then sputter-coated with gold palladium and observed under 15-25kV in a Jeol Scanning Electron Microscope (JEOL JSM 34C, Mfr Jeol Ltd, Tokyo, Japan).

The particles were grouped into diameter ranges 0.2-1, 1-5, 5-10 and 10-50μm ranges. Five representative scans from each of the capsules were taken at 2000 magnification, equivalent to 3177.78μm² of area over the filter. This was multiplied up to the total surface area of the filter, giving an approximate value for the number of particles found in the 20μm section.

The surface area of the capsule sections, from the wax sections, was quantified by image analysis. From rough measurements of the en bloc resections, the capsule depth was measured. Hence from the capsule “depth,” and surface area data an approximate volume of capsule was obtainable. The numbers of particles contained within this volume was calculated.

5.2.8.5
The superficial inguinal lymph nodes were removed from the animals. One animal also had the para-aortic nodes removed. The lymph nodes were photographed upon removal. Spleen and node processing was by standard methods for soft tissue sections of that size, for routine paraffin wax histology. Lymph node sections were digested in acid, as the capsules, for examination of particulate debris.
5.2.9 Basic Statistics

Blinded assessment was considered the most reliable means of unbiased assessment. Having received the data values, they were assessed for normality. This was in order to decide the policy for non-Gaussian or normal testing. Normality testing was conducted using the statistical package SPSS® (Statistical Package for Social Sciences). Normality plots with tests were used to display normal probability and detrended normal probability plots. The Kolmogorov-Smirnov statistic, with a Lilliefors significance level for testing normality, was used. (A Shapiro-Wilks statistic was also available for small samples). If a p value was less than 0.05, the data was deemed not normal, and would be analysed using non-normality tests. The goat data was considered independent, since different animals were being compared, hence the tests of choice were independent analyses using the Mann-Whitney U or the student’s t-test, quoting the two-tail P-value.

If the data was normal, an independent sample student’s t-test was used. This procedure tests the null hypothesis that the data are a sample from a population in which the mean of a test variable was equal in two independent groups of cases. It was similar to the analysis of variance (ANOVA) procedures, but restricted to a comparison of two groups.
### 5.3. RESULTS

In order to clarify the results they have been sub-divided into a number of sub-sections:

<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3.1</td>
<td>Femoral Head Data</td>
<td>Pre and post-operative surface parameters of the CoCr heads</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Head Penetration Data</td>
<td>The results from moulds of the acetabular cups to quantify wear.</td>
</tr>
<tr>
<td>5.3.6</td>
<td>Tissue Digestion</td>
<td>Capsule ring sections</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lymph nodes</td>
</tr>
<tr>
<td>5.3.3</td>
<td>Radiographic evidence</td>
<td>Acetabular loosening</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proximal femoral bone loss</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transverse femoral changes</td>
</tr>
<tr>
<td>5.3.4</td>
<td>Bone Histology</td>
<td>Acetabulum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proximal femur</td>
</tr>
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<td></td>
<td>Transverse femur</td>
</tr>
<tr>
<td>5.3.5</td>
<td>Soft Tissue Histology</td>
<td>Capsule ring section</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Capsule punch biopsies</td>
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<td></td>
<td></td>
<td>Lymph nodes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
</tr>
</tbody>
</table>

Table 5.4 Results Format
5.3.1 Femoral Head Data

5.3.1.1 Surface parameters

The tribological properties of roughened bearing surfaces have been discussed in Chapter 4. This demonstrated that roughened counterfaces produced large numbers of particles of 0.02-10µm in size. The wear factor could be controlled by changing the surface roughness.

Pre and post-operative mean values for surface roughness ($R_a$), average peak-to-valley distance ($R_{tm}$), and the mean spacing between peaks ($S_m$) were recorded over the superior, anterior, posterior and inferior surfaces of the femoral heads, as summarised in figures 5.6, 5.7, and 5.8 respectively. Data was recorded from four rough and four smooth post-operative heads, as well as the pre-operative parameters for rough heads. Scratching the femoral heads did not significantly (t-test: $P=0.144$) alter the outer diameters relative to the smooth controls.

![Figure 5.6 Average Roughness of Femoral Heads](image)

The graph in figure 5.6 demonstrates the large difference between the average roughness of the smooth and rough heads. Pre-operatively the heads were consistently rough over the surface.
Over the *in vivo* one-year period, the posterior, anterior, inferior, and superior regions of the head appeared to be rougher, but there were no significant changes (P values = 0.17, 0.30, 0.10, and 0.11 respectively).

The peak-to-valley values follow similar trends to the average roughness data, although no significance was found between the pre and post-operative values for the posterior, anterior, inferior, and superior regions (P values = 0.41, 0.32, 0.15, and 0.07 respectively).

---

**Figure 5.7 Mean Peak-to-Valley Distances of Femoral Heads**

**Figure 5.8 Mean Spacing Between Peaks of Femoral Heads**
The consistency in the pre-operative heads was confirmed, with no major differences in the distances between the peaks that corresponded to the mean readings, for either the smooth or the rough heads.

### 5.3.2 Head penetration rates

The data from the moulds indicated two centres for the femoral heads: where the head was originally placed, and then the worn position. This displacement gives a value for wear depth into the polyethylene cup.

Eleven hips were disarticulated for shadowgraphic analysis, R75, R76, R80, R83, R85, R87, R89, R90, R92, R94, and R95, with four smooth heads, and seven rough. Of the rough heads, there were three in the test group, R75, R76, and R80, and four in the control groups, R83, R85, R94, and R95.

Each mould was measured six times, and was re-positioned between measurements. The cup with the most inconsistencies during penetration recording was the cup from R83. There was a large variation in measurement noted in this cup, of approximately 30%, making the data from this cup unreliable. This was due to the very low wear that occurred in this cup. Excluding R83, the mean head penetration was 0.67mm (S.D.=0.13). For R83 the penetration was 0.20mm(S.D.=0.06). The large standard deviation, for one cup, relative to the rest, is indicative of the unreliability of the R83 data set.

To check that the penetration data was normal, the Kolmogorov-Smirnov statistic, with a Lilliefors significance level for testing normality, as well as a trended and untrended normal q-q was made. The low rate of R83 distorted the data significantly. If the head penetration data of R83 was included in a t-test for the comparison of control and e-PTFE groups, there was statistical significance (p=0.03), in the penetration rates.
There was significantly more wear of cups in the e-PTFE groups. If the R83 data was omitted from the calculations there was no difference ($P=0.29$), as demonstrated by the graph in Figure 5.9. This finding can be corroborated when considering the rest of R83 data.

![Graph showing wear of Rough Heads in e-PTFE and Control Groups](image)

**Figure 5.9 Wear of Rough Heads in e-PTFE and Control Groups**

The graph below details the penetration for the smooth and rough heads.

![Graph showing wear of Rough and Smooth Heads](image)

**Figure 5.10 Wear of Rough and Smooth Heads**

The penetration rates from the smooth heads was 0.52mm (S.D.=0.07). Having checked for normality and compared with a t-test, assuming unequal variance, there was a significant difference ($P=0.02$), between the smooth and rough head penetrations.
5.3.3 Tissue Digestion

5.3.3.1 Capsule Ring Sections.

The digestion of these sections produced particles shown in figure 5.11.

The digested capsules produced particles of the same size and shape as noted by other workers who have isolated wear debris in hip capsules around loose joint prostheses (Campbell et al. 1995, Hirakawa et al. 1996a, Kobayashi et al. 1997).

The digested capsules were 20μm thick and cut from the same block as the sections used for histology. For subsequent particle quantification, the surface areas of the cut sections were measured by image analysis. It was important that the capsule surface areas, for the e-PTFE and control groups, were statistically the same. Particulate number comparisons were being made between a variety of goats in different groups.
For the e-PTFE and control groups, a t-test of the tissue surface area to be digested indicated that they were statistically insignificant from each other (P=0.071). Hence valid comparisons can be made between the particles counted in the control and e-PTFE groups. Figure 5.12 shows the total numbers of particles calculated in the capsule. These values were calculated by the multiplying the number of particles found in the 20µm section with 1000. This would give an approximate indication of the numbers of particles located in the capsule, based on measurements of capsule depths between the acetabulum and femur, of 20mm, on average. An assumption is made that the distribution of the particles is linear throughout the capsule.

The total number of particles found in the control and test groups were the same (p=0.26). This supports the intention to test the e-PTFE and control groups with a particulate burden that was statistically not different in either group. Most particles were 0.2µm -0.9µm in diameter.

Figure 5.12 Numbers of Polyethylene Particles from Rough Heads only
The numbers of particles found in the capsules of rough and smooth heads were significantly different from each other \((p=0.000)\), as shown in figure 5.13.

Figure 5.13 Numbers of Polyethylene Particles

5.3.3.2 Lymph Node Digestion

No wear particles were found in the superficial inguinal lymph nodes. The one para-aortic node that was digested demonstrated particles of a similar nature to those noted in the capsule digestions.
5.3.4 Radiographic Evidence

5.3.4.1 In Vivo Radiographs

Every four months post-operative X-rays were taken. Figures 5.14a, b, & c are from R79, which was a control group animal.

Figure 5.14a

The radiograph in figure 5.14a is an AP radiograph of the right hip of R79 taken four months post-operatively. The femoral stem and the cement mantle of the acetabular component were visible. The cement mantle of the femoral component and the distal regions of the femur were less clear. In the proximal femur, adjacent to the lesser trochanter, a radiolucent area was noted. This lucency was observed in animals pre-operatively and not considered attributable to loosening in any way.
The dome of the acetabular component was in line with the ilio-pectineal line. A small radiolucent line was apparent at the cranial margin of the cup, which stops at approximately a quarter of the way around the cup.

The femoral cement mantle and femur were clear. Radiolucent lines were noted at both the cranial and caudal margins of the cup, each extending into Charnley zone 2 from either side. Relative to figure 5.14a, the cup had also medialised. Numerous osteophytes were also present.

At twelve months post-op, the most striking feature was the radiolucent region surrounding the cup. This radiographic presentation was typical in nearly all of the control group animals. Although less clear, the femur seems largely unchanged.
Figure 5.14c Radiograph of animal in control group taken 12 months post-operatively.

Figure 5.15a Radiograph of animal in test group taken 4 months post-operatively. The cup was well bonded.
Figure 5.15b Radiograph of animal in test group taken 8 months post-operatively. The cup remains well bonded.

Figure 5.15c
This radiograph demonstrates a well-bonded cup after 12 months in situ. The e-PTFE, on the posterior aspect was apparent as a thin radiopacity.
5.3.4.2 Radiography of en bloc pre-grind sections
The DEXA scanning of the pre-processed en bloc whole resections proved to be inconclusive. The acetabular osteolytic lesions noted in the radiographs were indistinguishable from the cement. There was also no delineation possible in the femora, from the cement and bone.

As noted in the materials and methods, the sections were attached to slides, cut to approximately 800μm and X-rayed before grinding down. The ‘pre-grind’ radiographs are shown in figures 5.16 and 5.17, with control and test respectively.

In the control goats, a clear radiolucent line was present on examination of the acetabular and femoral components. The femoral stem was taken as a ‘glancing’ section and was not precisely coronal. A proximal cement groove can be seen, as this was on the side of the implant.

Figure 5.16 Sectional Radiograph of Hip Joint in Control Group
There was marked osteolysis in the acetabulum with clear ‘scalloping’ in the acetabular wall. No such osteolytic lesions were noted in the femur.
The en bloc section from the test group in figure 5.17 demonstrated a clear radiolucent line around the cement of the acetabular component, but there were no osteolytic cavities as was found in the controls. The e-PTFE membrane was also present, with reactive bone formation on both the femoral and acetabular implants.

5.3.4.3 Radiographic Analysis of the Acetabular Cups

The serial radiographs of the acetabular cups demonstrate the extent of loosening. Two representative radiographs are presented below, from control and test groups as figures 5.18 and 5.19 respectively.
As noted in figure 5.18 the bone was crenated at the cement-bone interface. A single osteophyte was also clearly noted.

There was only a small radiolucent line along the inferior margin on this radiograph. There was also a nidus of intra-e-PTFE mineralised bone, as shown by the radio-opacity in the membrane.
From both the *en bloc* and the acetabular cup radiographs, measurements of the fibrous membrane thickness along the cup were performed and is shown for the rough heads in figures 5.20 and 5.21.

The greatest extent of loosening in the control cups are at the interfaces nearest the joint margin. The opposite situation was noted in the test groups, where the fibrous tissue was greatest at the apex of the dome, and very thin at the *interfaces*, nearest the joint space.

For each cup there are 36 data points. Twelve cups with rough heads have been compared. For the six cups in each group, there are 216 readings. When analysed using a Kolmogorov-Smirnov test, with a Lilliefors significance level for testing normality, the significance value for the 216 readings in both the control and test groups was less than 0.02. The data was not normal and hence not appropriate for Gaussian statistical tests. With non-parametric analysis, for independent samples, a Mann-Whitney U test produced a P value of 0.00.
Figure 5.21 shows the mean radiolucent line thickness for the control and test groups, and represents the cumulative data presented in figure 5.20.

![Graph showing mean radiolucent line thickness for control and test groups.]

Figure 5.21 e-PTFE reduced the extent of Loosening.

If the means are calculated for each animal, then the six values in each group compared to each other. The Lilliefors statistic was >2 indicating that these values were normal and appropriate for analyses with a student’s t-test assuming unequal variances. In a comparison of the means for each animal between the control and test groups, in a blinded assessment, the P value was 0.02.

The data from the smooth heads contain too few animal numbers to make statistical evaluation conclusive. Nevertheless the individual data points can be used, and for the sake of completion, the results from non-Gaussian independent analyses are presented in table 5.5.

<table>
<thead>
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<th>Experimental Groups Being Compared</th>
<th>P Values</th>
</tr>
</thead>
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<tr>
<td>Control Rough and e-PTFE rough</td>
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</tr>
<tr>
<td>Control Smooth and e-PTFE smooth</td>
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</tr>
<tr>
<td>Control Rough and Control smooth</td>
<td>0.0000</td>
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<tr>
<td>e-PTFE Rough and e-PTFE smooth</td>
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Table 5.5 Analyses of Smooth and Rough Heads
5.3.4.4 Proximal Femoral Radiography

The cement bone distances from the rough heads are summarised in figure 5.22.

The results show that the proximal regions of the femur were more vulnerable to loosening than the distal aspects. More measurements were taken on the lateral side, due to the geometry of the prosthesis/bone neck angle.
The absolute values, in mm for the group values from each of the data points proved to be non-Gaussian and were tested by Mann-Whitney U analysis. There were no significant differences in the cement-bone distances between the e-PTFE and control groups for both the lateral and medial sides (0.3975 and 0.1866 respectively), for the rough heads. The P values are summarised in table 5.6.

<table>
<thead>
<tr>
<th>Experimental Groups Being Compared</th>
<th>Bone Region</th>
<th>P Value</th>
</tr>
</thead>
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<tr>
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</tr>
<tr>
<td></td>
<td>Medial</td>
<td>0.1866</td>
</tr>
<tr>
<td>Control and e-PTFE. Both Smooth Heads</td>
<td>Lateral</td>
<td>0.0000</td>
</tr>
<tr>
<td></td>
<td>Medial</td>
<td>0.0000</td>
</tr>
<tr>
<td>Smooth and Rough Heads. Both e-PTFE</td>
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<td>0.0255</td>
</tr>
<tr>
<td></td>
<td>Medial</td>
<td>0.0000</td>
</tr>
<tr>
<td>Smooth and Rough Heads. Both Controls</td>
<td>Lateral</td>
<td>0.0000</td>
</tr>
<tr>
<td></td>
<td>Medial</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

Table 5.6

The second set of data that was available from the radiographic measurements were the values per animal. For the rough heads, this produced six values to compare in each of the e-PTFE and control groups. Having confirmed that the data was normal, a t-test that assumes unequal variance, comparing the e-PTFE and control groups on the medial and lateral aspects, produced p values of 0.71 and 0.30 respectively. This supports the results obtained from the raw data. In the groups containing the animals with smooth heads, there were only two in each group, hence conventional statistical analysis would give misleading information.
From the graph in figure 5.22, it was observed that there was a difference in the first five readings between the e-PTFE and control groups on the medial side. If the data points are analysed with a Mann-Whitney U test, a significance of 0.015 is obtained. This would indicate that the bone around the calcar was vulnerable to wear particle-induced osteolysis that was preventable by placement of the e-PTFE membrane.
5.3.4.5 Transverse Femoral Radiography

The statistical analysis of the percentage porosity along the femoral stems was carried-out using a t-test. There was no significant difference (P=0.21) between any 'osteolysis' in the transverse femora between the e-PTFE and control groups, as represented by figure 5.23.

![Figure 5.23 No difference between Groups in Distal Femora](image)

The results for comparisons from the smooth heads were unlikely to be meaningful since n=2, in each group.
5.3.5. Bone Histology

5.3.5.1 Acetabular appearance and Histology

If the hypothesis that birefringent wear debris caused the osteolysis at the bone-cement interface as noted in the radiographs, then it was important to establish the presence of wear debris histologically.

Upon macroscopic observation, the bone osteolysis was confirmed in the resin sections, as demonstrated below by figure 5.24.

Figure 5.24 Control Cup with Roughened Head, showing large cement-bone interfacial gap.
The previous figure can be compared with figure 5.25 below, clearly showing incorporation of the white e-PTFE membrane with bone.

![Figure 5.25 e-PTFE sealed acetabular interface, by GBR.](image)

The bone interface was smooth, and not showing the crenated appearance associated with the control group. The lack of medialisation was corroborated by the continual integrity of the ilio-pectineal line. Reactive bone formation adjacent to the e-PTFE membrane was seen in all test animals, demonstrating Guided Bone Regeneration.
Figure 5.24 confirms that the thickness of the osteolytic fibrous membrane was greatest at the margins of the cup, where it was initiated. This was not the case for the e-PTFE group, where intimate cement-bone macrointerlock was achieved, as shown by figure 5.26.

Figure 5.26 Cement-Bone interface at the cup margin for e-PTFE group showing cement and bone in close approximation (X100)

Figure 5.26 can be compared to the figure 5.27, taken at the same location, and magnification. The cement-bone interfaces are at either side of the picture, showing the fibrous tissue ingression, between the cement and bone. Swollen macrophages are observed centrally, interposing the parallel collagen fibres and the acellular tissue on the right and left respectively. The bone interface is visible on the far right, as the densely stained tissue.
Figure 5.27 Fibrous tissue Interposing the Bone and Cement at the Cup Margin for the Control groups (X100)

The same location, taken with a polariser, is shown in figure 5.28.

Figure 5.28 Polarised image of the Fibrous Tissue Interposing the Bone and Cement at the Cup Margin for the Control Groups (X100)
The large quantity of intra-cellular birefringent material was apparent. There were also a numerous birefringent particles in the extra-cellular spaces.

A clear resorption wedge was noted, progressing to the cup apex, with osteolysis, as demonstrated in figure 5.29, and detailed in figure 5.30.

In association with the osteolytic areas were noted macrophages, shown in figure 5.31. When this image is polarised, intracellular birefringence is seen, as illustrated in figure 5.32.

Figure 5.29. Osteolytic wedge in control group (X40)
Figure 5.30. Osteolytic detail from resorption wedge in control group (X200)

Figure 5.31. Macrophage detail from osteolytic wedge in control group (X200)
No such resorption pits in association with wear debris was found in any of the test groups. The interface between the apex of the cup and the bone contained neither macrophages nor wear debris, as demonstrated in figure 5.26.

At the e-PTFE-bone interface, there was mineralised bone formation up to the membrane, as shown by the back-scattered image of figure 5.33.

Figure 5.32 Macrophage detail showing intracellular birefringence (X200)
Figure 5.33 Interface between e-PTFE and bone in acetabular section. This shows intimate lamellar bone-e-PTFE contact.

This image was at the junction of the membrane/bone bond where the glue originally had been applied. There was shown to be no differences in the bone bond overlying the membrane. Consequently, the presence of the glue did not affect the final membrane-bone bond, as well as allowing bone growth over the end of the e-PTFE insertion as demonstrated by figure 5.25: Guided Bone Regeneration.

A 'window' into the matrix of the membrane in visible near its mid-point, showing a view of the inter-nodal connecting fibrils

Histologically osteoblastic-like cell penetration into the matrix was found throughout the bone-e-PTFE junction (Figure 5.34). Although in the majority of cases, there was no intra-e-PTFE ossification as formal mineralised bone deposition.
There was intra-e-PTFE cell incorporation in both the central and peripheral regions throughout the e-PTFE membrane. Hence the use of glue attachment seemed not to affect the bone-cell response to the presence of the membrane. In the areas where cells had not colonised the e-PTFE pores, they were in direct contact with the membrane.

At the other side of the membrane, fibrous tissue had grown throughout the pore matrix. As noted with osteoblasts on the bone surface, fibroblasts could be located throughout the membrane matrix adjacent to the overlying soft-tissues.

The figure below demonstrates how the two outer porous layers allowed mutually exclusive cell types to grow throughout the matrix of the e-PTFE membrane.
Figure 5.35 Two cell populations, bone and fibrous tissue growing into either side of the e-PTFE membrane (X40)

Adverse reactions to the butyl-cyanoacrylate and e-PTFE were noted in neither the soft-tissues nor the bone.

Figure 5.36 shows the overlying soft-tissues penetrating the e-PTFE matrix, in a similar fashion to the bone.
5.3.5.2 Proximal Femoral Histology

Birefringent particles were found at the interface between the soft tissues of the hip capsule and the proximal femur. The control groups presented in a similar fashion as the acetabular component. A 'resorption wedge' progressed into the bone as demonstrated in figures 5.37, and the polarised figure as 5.38. Macrophage detail, normal and polarised are represented by figures 5.39 and 5.40 respectively.
Figure 5.37 Resorption wedge of femoral component in control groups (X40)

Figure 5.38 Resorption wedge of femoral component in control groups (Polarised) (X40)
Figure 5.39 Swollen Macrophages at the Proximal End of the Medial Femoral Component in the Control Group (X200)

Figure 5.40 Macrophages containing Birefringent Material at the Proximal End of the Femoral Component in the Control Group (Polarised) (X200)
Figures 5.37 - 5.40 were taken at the medial side of the femur, adjacent to the calcar. The resorption wedge was not found further than approximately 5mm distal to the shoulder of the femoral component. At the cement-bone interface in these more distal regions, no such particles were noted in the control or the test groups, as shown in figure 5.41.

The test groups did not show the presence of this resorption wedge, only collagen and very much fewer macrophages that did not contain any birefringent material.

In the cement and bone interface distal to the resorption wedge, parallel fibres of collagen and fibrous tissues were noted. Swollen macrophages, in the quantity found in the acetabular component, were not noted, as shown in figure 5.41.

Figure 5.41 Fibrous tissue between the cement and bone in the Proximal Femur as noted in both test and control groups (X200)
Except in the proximal medial aspect of the femoral component, there were no appreciable differences in the histological profiles between the control and test groups.

The shoulder of the titanium proximal femoral component and the e-PTFE bond was in very close approximation. This indicated that the interface was well established as demonstrated in figure 5.42. The e-PTFE had been glued to the titanium during surgery, using the same butyl-cyanoacrylate.

Figure 5.42 e-PTFE and titanium in close approximation on the proximal femoral component, at the shoulder
5.3.5.3 Transverse Femoral Histology
5.3.5.3.1 Undecalcified Femoral Histology
The histological picture in the transverse femoral components was similar to the distal and lateral regions of the proximal femora. There were not many macrophages at the interface, and very little birefringent material was found in the fibrous membrane. At low power, an intimate bond was noted between the cement and bone as in the macroscopic figure 5.43.

Figure 5.43 Well-bonded cement mantle in control group

When the cement-bone region is examined microscopically, a collagen layer is found in between the cement and bone, as in figure 5.44.
5.3.5.3.2 Decalcified Femoral Histology

Decalcified histology did not contain any cement or metal, and confirmed the observation made in the undecalcified sections. No wear debris was noted at the interface.

Figure 5.44 Collagen Layer in between Cement and Bone in Transverse Femur
5.3.6 Soft Tissue Histology
5.3.6.1 Capsule Ring Sections

The 'ring biopsies' were made up of intra- and extra-capsular structures. Extracapsular muscle fibres were noted running both parallel and perpendicular to the tissue plane, representative of both the anterior and posterior muscle groups. The intra-capsular structures can be divided into three groups, as represented by the idealised schematic of figure 5.45.

Located peripherally were birefringent collagen-rich connective tissues. The fibres were orientated parallel to the implant surface forming a capsule of concentric layers around the implant. Macrophages were interposed between the collagen sheets, and located throughout the capsule biopsy. The capsules surrounding the implants with rough heads, had a larger number of macrophages, although this was not formally quantified.

Localised throughout the capsule were collagen, swollen, macrophages, and necrotic tissues containing birefringent material. Details of this material could not be resolved at the level of the light microscope.
Section demonstrating swollen macrophages, and collagen sheets (Figure 5.46), with birefringence when polarised (Figure 5.47).

Figure 5.46 Swollen Macrophages and collagen (X100)

Figure 5.47 Intracellular Birefringence with collagen birefringence (X100)
Figure 5.48 Macrophages in hip capsule tissue (X400)

Figure 5.49 Birefringence within Macrophages (X400)
The macrophages present in both groups contained a large amount of submicron birefringent material. Figures 5.48 and 5.49 are H & E stained, with the latter figure polarised. These histological images correlate to the findings of co–workers (Baslé et al. 1996, Hirakawa et al. 1996a).

Sections R75, R89 and R90 contained fragments of e-PTFE, approximately 4-7mm in length, surrounded by macrophages. In association with these fragments where noted smaller particulate (100-50um). However the quantity of this was very small indeed.

By plasma etching the capsule sections and examination of the intracellular macrophage detail, by SEM, the holes left by the polyethylene were demonstrated (Figure 5.50).

Figure 5.50 Scanning Electron Micrograph of a Plasma etched Resin section showing macrophages with vacuoles which contained UHMWPE material. Many wear particles were pulled-out of the section during preparation.
Benz et al. 1994 showed that preparation of sections for TEM pulled-out the polyethylene particles from the macrophage cytoplasm.

5.3.6.2 Capsule Punch Sections
The sections were isolated areas of the capsule sections and demonstrated the features that were described for the ring biopsies.

5.3.6.3 Lymph Node Histology
The superficial inguinal lymph nodes did not show the presence of birefringence on histological examination. In R75, whilst dissecting to the aorta, the para-aortic lymph nodes appeared swollen. These showed wear debris within macrophages, as shown in figure 5.51.

Figure 5.51 Macrophages & Birefringence, surrounded by Lymphocytes
Images can be compared directly to the findings of Baslé et al. 1996, Hicks et al. 1996, Shea et al. 1996).

5.3.6.4 Spleen Histology
No birefringence was noted in any of the sections taken from the spleen.
5.4 DISCUSSION

5.4.1 Femoral Head Data
The importance of achieving a reproducible and consistent femoral head roughness and morphology was essential to ensure that the wear and effects of wear debris of different goats could be compared with each other. The level of goat activity with the ensuing particle generation cannot be precisely accounted for. To investigate that goat activities will not be significantly different from each other was not undertaken, but an absolute minimum level of activity was imposed. Short of treadmill exercising, other methods for accounting for activity levels are time and labour intensive.

The results demonstrated that the anterior, inferior and superior aspects of head roughened, but not significantly. This could have been attributable to third body wear, but unless the posterior head region was an aberrant reading, it was difficult to speculate what makes this area less susceptible. The femoral component was inserted with approximately 15° of anteversion, making the posterior region in close contact with the cup. This would make it susceptible to polishing effects. However the cup was also inserted with 15° of cover, hence the superior margin would be the most load-bearing (Yamaguchi et al. 1997), so why should this scratch more? A potential theory to explain these results could be a variation in the distribution of particles that elicit third body wear. This however seems unlikely in congruent bearing surfaces that are in motion.
5.4.2 Acetabular Cup Wear and Femoral Head Penetration

Accounting for the head penetration was important since it was essential to know that the non-e-PTFE and e-PTFE groups have been tested using a similar particle burden. Head penetration rates are a guide to the extent of wear, and hence the quantity of particulate debris. It is important to appreciate that the method takes into account the combined effects of creep and wear.

Human wear rates in the recent studies, using more accurate post-mortem analysis, have been shown to be 0.07mm/year (Sychterz et al. 1996). This data is taken over years, reducing the contribution of deformation, unlike the goat cups that were in vivo for one year, where deformative effects would have been greatest. The wear rates from the rough heads were 0.67mm/year.

Including the low wear rate of R83, a control goat, significantly more wear was produced in the e-PTFE groups, than the controls. The actual relevance of this is that, based solely on this data, the e-PTFE goats were unintentionally exposed to more wear debris than the control groups. If there was to be an unavoidable discrepancy in the wear burden presented to the two groups, for obvious reasons greater wear in the test group, rather than the control group, would be a more favourable situation. The low wear in animal R83 may have been possibly due to the low activity levels. The penetration data is confirmed as being accurate, since the numbers of wear particles in the hip capsule tissue was equivalent to the smooth head groups, and not a roughened head.
The wear penetration rates for the smooth heads, relative to human data, was high. This may however be artefactual, since the human retrieval analyses are made after more time than 1 year in vivo. It was possible that in the first year or thereafter, head penetration was due to both to plastic deformation of the polyethylene as well as wear. The significant difference (P=0.022) between the rough and smooth heads was as expected. In retrospect, larger numbers of smooth heads would have been ideal for statistical comparisons made between the rough and smooth heads with more credence.

5.4.3 Radiographic Evidence
5.4.3.1 Acetabular Radiographic Discussion
The in vivo radiographs demonstrated clear bony erosion and new sclerosis. The 'resorption wedge' progression occurred at the margins of the cup and migrated towards the apex. This finding is in agreement with the published literature on the mechanisms of acetabular cup loosening (Schmalzried et al. 1992b, Jasty et al. 1997). No such loosening was noted in the e-PTFE groups.

The value of serial radiographs demonstrates that the loosening action was progressive. This was in keeping with the action of wear debris and cup loosening. A resorption wedge was needed to facilitate access for the debris around the whole of the cup. In previous studies cracks have been seen in the femoral cement mantle but not surrounding the polyethylene of the acetabular cup. Therefore wear debris appears to access the cement-bone interface (Jasty 1997).

The radiography of the sections demonstrated clear osteolysis in the wall of the acetabulum. These were apparent as "punch" lesions at the bone-cement interface, with clear osteolysis progressing medially. The graph summarising the cement-bone distance, or the fibrous tissue thickness, shows clearly that the greatest distance was apparent at the margins of the cup. This confirms the progressive nature of wear particle induced osteolysis in the cup.

The test groups do not show this finding, indicating that the effect of the wear particles has been prevented by the membrane.

In an assessment by an independent reviewer, the blinded radiographs were put into osteolytic and non-osteolytic groups. Except R83, all of the control and test groups, in test and control groups, were selected correctly. The finding is clear.
The greatest thickness, in the e-PTFE group, of fibrous tissue was noted at the apex of the cup. At the cup margins, the e-PTFE membrane was integrated with bone. Ideally, this dome-detachment would not be present. Possible reasons for this radiolucency, in Charnley zone two, may be the cement polymerisation exotherm, lack of macro-interlock, or poor cementing technique. In the goat, the intra-ligamentum teres artery appears to be more vascular than in the adult human situation, and leads to bleeding that prevents an adjacent cement-bone bond. The most likely reason was the morphology of the acetabular wall. There was very little cancellous bone, and the reamed surface of the acetabular wall, was smooth. Three drill holes were made, for fixation, and in retrospect, more cement inter-digitation ought to have been ensured.

The significance testing at the bone-cement interface of the acetabular component was important. It was an essential statistical process to check for normality in the data obtained from all experimental research, such that appropriate statistics are subsequently used. For both the parametric and non-parametric data, significance was obtained. This confirms the observational findings, which are in themselves evident.

The nidus of intra-membrane ossification was interesting. It must be stated that this was not a generalised finding in the e-PTFE membrane. In retrospect, an ideal membrane design would facilitate more generalised intra-membranous ossification. Although it may be possible, that intra e-PTFE membranous ossification takes more than a year to occur. Further work would clarify this position.

The bone-growth around the end of the membrane and then over its surface was an unexpected positive finding, indicating that guided bone regenerative concepts can be used to seal the interfaces of the implant bone, both mechanically and biologically.
Doubts regarding the reliability of the data from the smooth heads have been expressed because there were too few animals in that experimental group. Based on wear, there was little reason to believe that there would be a significant difference between the control and e-PTFE smooth groups. The data did not demonstrate this, indicating there was more wear, of the smooth heads, in the control hips than the e-PTFE sealed hips, which themselves wore significantly less than the rough heads.

5.4.3.2 Femoral Radiography
The analysis of the fibrous tissue thickness demonstrated a difference in the proximal 5mm on the medial side between the e-PTFE and control groups. Although no difference was found down the proximal stem in the rest of the measurements.

This proximal loosening may have occurred by similar mechanisms as evident in the acetabular component. This is generally not thought to be actual mechanism responsible for long term loosening of the femoral component in the human context. It is possible that this system produced loosening in this form for two reasons. (1)The hip capsule was flooded with particles at an artificially accelerated rate, and so caused osteolysis wherever bone was present. (2)The femoral debonding mechanisms, and formation of wear debris-conducting pathways, were unable to form in the year that the prostheses were in place.

Perhaps the lack of formation of these conducting pathways may have explained why there were no differences in the e-PTFE and control groups in the distal regions of the stem. The actual extent of loosening, for both groups, was only a very thin fibrous tissue membrane. When the control loosening graphs for the femoral and acetabular components were compared, it was clear that only a minority of femoral fibrous thicknesses, were larger than 1mm. This was in marked contrast to the control group in the acetabular component.
It is probable that the majority of the femoral component did not have the original hypothesis tested. A longer-term study may have allowed the required femoral osteolysis to occur.

The loosening that was found in the proximal component occurred by similar mechanisms as noted in the acetabular component.

For the examination of the non-parametric data Mann-Whitney-U analysis has been used since it is a powerful test that is applicable for independent samples. As with most non-parametric tests it does require a large number of data points to be reliable. Experimental insufficiencies were the small numbers of animals in the smooth head groups. Initially they were never considered as being a group within themselves. In retrospect more data would have been of benefit. However as with many cohort studies, cost was rate limiting.

In both the lateral and medial locations, for the control and e-PTFE animals with the smooth heads, no significant differences were noted as expected. Both smooth heads for e-PTFE and controls produced significant differences, with a greater fibrous membrane thickness in the control groups. In the e-PTFE groups for smooth and rough heads, there were significant differences between the cement-bone distances in each group.
5.4.3.3 Transverse Femoral Radiology

The data obtained from the transverse femora indicated that the use of e-PTFE did not have any significant effect on 'loosening' relative to the controls. The assessment of loosening needs to be queried for the transverse femora. In normal bone, the delineation between cancellous and cortical bone is not absolute. Using the method for loosening assessment in this study, it was apparent that the radiographic 'pores' within cancellous bone, were difficult to distinguish from osteolytic lesions. In terms of all of the radiographic material in general, further analyses of the en bloc sections using CT would have been of benefit. This would have aided the 3-D visualisation of the hip joint.

5.4.5 Soft Tissue Histology and Digestion Data

Obtaining accurate values with respect to particle size and morphology was essential for the development of a realistic loosening model.

Bone, soft tissues and particulate debris interactions were mimicked in an accelerated process. The actual mechanisms were simulated at the initiation of the process that goes on to effect them. The complexity of those processes that carryout loosening are currently, far from understood. The production of realistic loosening debris is therefore quintessential to the progression of loosening.

Most animal models which generate wear particle induced osteolysis do so by the introduction of a bolus of particles during the initial operation (Hasselman et al. 1997, Shanbhag et al. 1997). Steady-state in vivo production of realistic debris is a more appropriate design, both conceptually and practically.
The capsule digestions were essential in quantifying the amount of debris, as well as the debris morphology. The literature that describe debris morphology pictorially show a marked resemblance with the polyethylene debris noted in the digestions (Margeviucius et al. 1994, Shanbhag et al. 1994, Campbell et al. 1995, Baslé et al. 1996, Hirakawa et al. 1996a, Wolfarth et al. 1997). This is an essential finding.

It is appreciated that the values given for particles contained within the capsules are approximate. There are number of assumptions, that affect these results directly. These include: all of the capsule was biopsied, particles are not lost during de-waxing, dehydration and subsequent digestion of the sections, all of the capsule particles appeared on the filter, the surface area measurements were representative of the capsule distribution, and that the capsule thickness from the cup to the femur was 20mm. In spite of these assumptions, the data can be compared to each other. The ring biopsies were treated in the same way, and hence have at least a relative value, for data comparisons to be made.

The finding that there were no significant differences between the numbers of particles counted over the filters, and hence in the capsules, was obviously very important.

It is not surprising, that the most numbers of particles were in the 0.2-0.9μm range. As stated in the results there were no significant differences between the number of wear particles in the control and e-PTFE groups. Although as the graphs show, there was a trend that more particles were produced from the e-PTFE groups. Again, this was the preferable situation since the e-PTFE ought not to be tested with fewer particles than the controls.
The wear particle number produced from the smooth heads was not surprisingly, less than the rough heads. The polyethylene debris in the capsule was accounted for. There are obviously large quantities of debris also located throughout the hip joint, as well as the reticular endothelial system of the lymphatic supply to the pelvis and femur. This study did not attempt to quantify the amount of metallic debris that must have been present in the hip capsule.

Immunohistochemical analyses could have also been undertaken to further characterise the cellular profile in the hip capsules of the test and control as well as the smooth and rough heads.
5.5 Summary and Conclusions

The aim was placement of an e-PTFE membrane that would form a physical, as well as a biological seal of the acetabular and femoral bone-implant interfaces. A loosening total hip arthroplasty model was developed that was comparable to the radiographic and histological presentations of loose total hip replacements in humans. The implants were left in situ for one year.

No adverse soft or bone tissue reactions were noted from the e-PTFE membrane as well as the butyl-cyanoacrylate used to attach the membrane to the bone surfaces. The cement bone distances were assessed in the acetabular and proximal femoral components. No differences were noted in the proximal femur between the control and test groups. The cement-bone distance of the acetabular component of the test groups was significantly less (p=0.02) than the control groups. This method has been shown to prevent loosening of the acetabular component.
Chapter 6 Summary and General Discussion

6.1 Experimental Summary

The aim of this study was to prevent sub-micron wear debris reaching the interfaces of the acetabular cup and femoral component using a partially occlusive e-PTFE membrane. It was hypothesised that this membrane would initially act as a physical seal, which would become incorporated with bone and soft tissue, to form a secondary biological seal. The membrane would prevent debris migration to the bone-implant interface, and preclude wear particle induced osteolysis and consequential implant loosening. In humans aseptic loosening generally occurs after the first decade, by wear debris induced osteolysis and mechanical factors, such as stem debonding from the cement associated with joint loosening.

In this study it was not feasible to use this membrane in humans, hence an animal model was selected. The biocompatibility of the membrane and the glue was initially assessed using in vitro experiments. Over only 24 hours osteosarcoma cells, which were seeded onto e-PTFE membranes, proliferated at 50% the rate of cells on Thermanox control surfaces. The use of e-PTFE, in conjunction with butyl-cyanoacrylate reduced the rate of proliferation to approximately 20% of controls. This implied that the e-PTFE membrane was not optimally biocompatibility, when used in conjunction with the glue. However, the effect of reduced biocompatibility was not detected in an in vivo rabbit study since both soft tissue and bone integrated with the e-PTFE membrane, including the sites of glue attachment.
Chapter 6

The rabbit study also demonstrated that e-PTFE facilitated bone growth more rapidly in a protected site than an unprotected site. The second component of the rabbit study demonstrated that e-PTFE could aid the osseo-integration of a titanium screw. These two experiments demonstrated that the exclusion of fibrous tissue by the e-PTFE membrane enhanced growth in long bones. The use of these materials indicated that a secondary seal is formed when the membrane was used in a Total Hip Replacement application.

In order to test sealing the bone-implant interface, a total hip arthroplasty large animal model was developed. The aims of this model would be to generate loosening around a THR after one year. The loosening model would present with the same histological and radiological features as the human context after the first decade.

The heads of the femoral components were roughened to a known degree in order to produce an excess of polyethylene debris to accelerate implant loosening. Degrees of roughening had been investigated in a series of wear tests. The rough head produced particles that were similar in size and shape to those seen in the loose hips of humans after the first decade of the implant lifetime. It was hypothesised that this model would simulate an accelerated femoral and acetabular loosening.

Loosening was assessed by measurement of the bone-cement distance on radiographs. Loosening was attributable to osteolysis caused by wear debris because histological investigation revealed birefringent material within macrophages, consistent with the appearance of polyethylene wear particles under the light microscope.
Upon retrieval there was complete acetabular loosening and partial femoral loosening. It was proposed that aggressive femoral loosening was not observed because the one year period in situ was not long enough for the mechanical factors that facilitate femoral debonding and the formation of debris pathways, to take place. However since it is acetabular loosening that is considered more of a threat to the survivorship of total joint prostheses, than the loosening of the femoral component, this model is directly applicable.

To check that the control and test groups had been tested with the same particulate burden, wear penetration of the femoral heads was measured, as well as particle numbers in the peri-prosthetic tissues. Because of one goat, with very low wear, in the control group, analyses showed that there was more head penetration in the test group, than the control group. This indicated the test groups were being tested under harsher conditions. Analysis of the particulate numbers revealed that there were no significant differences in the particles produced for the e-PTFE and control groups. Most of the particles were under 1μm and had a similar morphology to those observed in tissues from around THRs.

The e-PTFE membrane was attached to the prostheses and glued to the bone, preventing the entrance of particulate debris to the acetabular bone-cement interface. Loosening was assessed by measurement of the radiolucent line in the acetabular and proximal femoral component. Compared with the control group in a blinded assessment, the e-PTFE significantly prevented acetabular loosening (P=0.02). Analysis of the medial 5mm of the proximal femora, adjacent to the calcar, demonstrated that the presence of the membrane significantly reduced the cement-bone distance in this region.
Wear debris was not found in interfacial tissues of distal femur in both groups. It was suspected that the feasibility of e-PTFE in the prevention of wear debris reaching the interface of the femoral diaphysis was not tested.

The sealing effect would not have accounted for the wear developed by the stem interfaces since these are located distal to membrane placement. Particulate debris was not found in the interfacial tissues of the femoral diaphysis. This maybe due to limitations in the model, implant insertion problems, or possibly that stem wear does not make a large contribution in the first year of the joint.

To summarise, we prevented wear particle induced osteolysis in the acetabular component of a loosening goat model using an e-PTFE membrane to seal the bone-cement interface.
6.2 CONFOUNDING FACTORS

The identification of confounding and limiting factors are extremely important for proper interpretation of the results and planning future research.

Anticipation of potential confounding factors averts experimental flaws. As appreciated by Charnley, osteolysis shares certain similarities with infection, and hence he developed the 'ultraclean-air operating theatre.' Without the swabbing from goat hips upon retrieval, it is possible, though nearly infeasible, that the osteolysis associated with the control hips was in fact infection.

Confounding factors may be avoided through study design and appropriate data evaluation. The ideal is to circumvent confounding factors during experimental set-up, leaving a straightforward and clear data analyses policy. Variables such as age and sex of the goat population used for this study were kept constant. Goat allocation to control and test groups was randomised. Data stratification was avoided by attempting to account for potential confounders before measurements were required. The measurements of fibrous tissue thickness, for the acetabular cups, incorporated both animal groups, including R83 that was deemed a low activity goat. Although correct statistics could be used to justify exclusion of the aberrant goat results from the study, significance was still achieved with the R83 data included. This adds weight to the overall conclusion, and demonstrates a clear difference between the control groups, and the goats implanted with the e-PTFE membranes.

Fluid pressures (Van der Vis et al 1997 & 1998, Aspenberg et al 1998) may have been prevented by the action of the membrane limiting the exposure of the interface to the joint pressures. The membrane is permeable to fluid, but will delay the 'hydraulic' force transmission.
6.3 FURTHER WORK

Having demonstrated the feasibility of the sealing methodology and the safety and effectiveness of the materials used, the next appropriate stage would be to conduct a human trial for sealing the acetabular component. It is important to adapt the e-PTFE to enable its insertion with an uncemented cup.

Solving the problem of wear particle generation will prevent wear particulate induced osteolysis. Hence the 'seal' used to prevent the migration of wear debris would be used solely to aid osseo-mechanical integration of prostheses, since the presence of wear particles would not be significant.

The title for this thesis is "Sealing the Bone-Implant Interface around Total Hip Replacements using Guided Bone Regeneration." The role presented was to facilitate longer-term implant fixation by preventing the adverse effects of sub-micron particulate matter on the bone at the implant-bone interface.

The use of this membrane in orthopaedic surgery is also an overall aim of the study. Aiding incorporation of revision prostheses following proximal bone loss, as well as osseo-mechanical incorporation of massive endoprostheses following primary bone tumours, are fields which merit further work using the e-PTFE membrane.

Further projects encompass non-union treatment, enhancing bone repair in the complicated fracture setting, as well as the maintenance of biochemical factors within a particular healing site. There are further applications to bone transport work, as well as increasing the size of bone areas to be harvested for autogenous bone grafting. A longer-term goal could include the growth of "bone cylinders," either along prostheses or independently.
The rate-limiting step for further work is not the regenerative capabilities of bone, but the willingness of orthopaedic researchers and surgeons to use them.

It is ironic that a derivative of the material, Charnley deemed to wear at an unacceptably high rate, could be used in an alternative setting to prevent the effects of particulate matter produced from the articulation.

Both cement and hydroxyapatite were first used in dental therapies before they were utilised in orthopaedic surgery. These two concepts are now commonly used in total joint arthroplasty. Guided bone regeneration may be the third.

6.4 CONCLUSION
The use of e-PTFE to seal the interface, in an experimental total hip arthroplasty model, prevented loosening of the acetabular component.


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