Natural History of Bone Response to Hydroxyapatite-Coated Hip Prostheses Implanted in Humans

P. Frayssinet
Bioland, Toulouse

D. Hardy
Hopital St Pierre, Bruxelles

J. S. Hanker
University of North Carolina

B. L. Giammara
University of Louisville

Follow this and additional works at: https://digitalcommons.usu.edu/cellsandmaterials

Part of the Biomedical Engineering and Bioengineering Commons

Recommended Citation
Available at: https://digitalcommons.usu.edu/cellsandmaterials/vol5/iss2/2

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Cells and Materials by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.
NATURAL HISTORY OF BONE RESPONSE TO HYDROXYAPATITE-COATED HIP PROSTHESES IMPLANTED IN HUMANS

P. Frayssinet1*, D. Hardy2 J.S. Hanke3 and B.L. Giammara4

1Bioland, 132 Rte d’Espagne, Toulouse, France,
2Sce de Chirurgie Orthopédique, Hôpital St Pierre, Bruxelles, Belgique,
3Biomedical Engineering Department, University of North Carolina, Chapel Hill, NC, USA,
4Analytical Electron Microscopy Department, University of Louisville, Louisville, KY, USA

(Received for publication July 21, 1994 and in revised form July 11, 1995)

Abstract

A series of 15 autopsied femurs containing hydroxyapatite-coated (HA-coated) prostheses was analysed histologically. Their implantation time ranged from 5 days up to 3 years. The coating thickness of some prostheses and the percentage of the coating in contact with bone at different levels were evaluated using an image analysis device. After the newly formed bone tissue had become mature, several bone morphotypes were identified at the coating contact. From the proximal to the distal part of the prosthesis, bone morphotype was denser and the percentage of the coating surface in contact with bone increased. Several stages in the prosthesis osseointegration were evident. The early bone formation was characterized by a direct ossification forming a trabecular bone in the bone marrow cavity between the prosthesis and the endosteum. A few weeks after implantation, osteoblasts, differentiating from the loose connective tissue which invaded the bone marrow cavity, synthesized an osteoid matrix on the coating, forming an immature bone. During the maturation, several morphotypes appeared and the bone remodeling also involved the ceramic coating causing resorption and ingrowth inside the coating.

Key Words: Hydroxyapatite(HA), HA-coating, plasma spray, ceramic, hip-prosthesis, osseointegration, coating degradation, remodeling.

Introduction

Bioactive materials are defined as materials which are designed to elicit or modulate biological activity [41]. With regard to bone replacement materials, however, bioactivity is directly related to the term bone bonding. Bioactive materials include hydroxyapatite (HA)-ceramics which were shown to bond to bone tightly [26, 27]. The mechanism of bone bonding is based on the formation of an apatite layer on the ceramic material suggesting a type of chemical bonding [8, 35].

This thin ceramic layer gives the biocompatibility of HA to the coated alloy without modifying its mechanical properties. Bone can form in close vicinity to the material and can be apposed on the ceramic achieving the locking of the prosthesis stem inside the femur bone marrow cavity.

HA-coatings obtained by a plasma-spray process can have different characteristics depending on the plasma-spray parameters and the powder characteristics. From the abundant literature published [12, 13, 14, 28, 29, 34] on the correlations between calcium-phosphate characteristics and the tissue behaviour at their contact, it is difficult to predict the osseointegration of this kind of material in human bone.

A lot of questions have been raised concerning the outcome of the material and the bone-material interface in the long term when implanted in humans. Cell degradation of coating has already been described [17] and osteolysis was attributed to the emission of calcium phosphate debris by the coating [3, 4].

Although HA crystals were shown to be a possible irritant [38], Wang et al. [40] demonstrated that phagocytoble HA-particles (5 μm diameter), implanted in bone harvest chambers in the tibial metaphysis of rabbit, did not impair bone formation. Bloebaum [3, 4] found calcium phosphate particles inside polyethylene inserts used with HA-coated prostheses, and evoked the risk of third body wear induced by the presence of HA-particles at the articulating surfaces level. However, calcium phosphate crystals have been found in polyethylene inserts which were not in contact of HA-coated materials [20].
Table 1. Patient data.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age (years)</th>
<th>Male or Female</th>
<th>Implantation time</th>
<th>Sepsis (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83</td>
<td>F</td>
<td>6 months</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>81</td>
<td>F</td>
<td>6 weeks</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>74</td>
<td>F</td>
<td>6 months</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>F</td>
<td>7 months</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>85</td>
<td>F</td>
<td>26 months</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>89</td>
<td>F</td>
<td>24 months</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>83</td>
<td>F</td>
<td>3 months</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
<td>M</td>
<td>3 weeks</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>90</td>
<td>M</td>
<td>9.5 months</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>75</td>
<td>M</td>
<td>5 days</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>80</td>
<td>M</td>
<td>1 month</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>75</td>
<td>F</td>
<td>9 months</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>76</td>
<td>M</td>
<td>7 months</td>
<td>N</td>
</tr>
<tr>
<td>14</td>
<td>82</td>
<td>M</td>
<td>1.5 months</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
<td>M</td>
<td>36 months</td>
<td>N</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of coatings of the prostheses (Coral, Landos, France) examined in this study.

<table>
<thead>
<tr>
<th>Plasma-sprayed powder</th>
<th>HA-coating</th>
<th>HP-coating</th>
<th>HA-coating thickness</th>
<th>Trace elements</th>
<th>Tensile strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>more than 97% HA</td>
<td>Majority of HA, amorphous phase, TCP/CaO (traces)</td>
<td>60 ± 10%</td>
<td>150 ± 50 μm</td>
<td>As &lt; 3 ppm; Cd &lt; 5 ppm; Hg &lt; 5 ppm; Pb &lt; 3 ppm</td>
<td>&gt; 35 Mpa</td>
</tr>
</tbody>
</table>

In order to study the osseointegration of HA-coated prostheses, we have analysed, using histological means, a series of 15 identical HA-coated hip prostheses implanted in humans for period from 5 days to 3 years. The upper femur containing the prosthesis was retrieved post mortem and was processed for histological analysis.

**Materials and Methods**

Fifteen patients (Table 1) implanted with an HA-coated hip prosthesis and having died for reasons that were not related to the surgery (except for the five-day implanted patient who died of cardiac failure in the post-operative period) were autopsied and the upper part of each femur, which was implanted with hip prosthesis, was removed for histological analysis.

The hip implant consisted of a fully coated self-locking stem of Ti-6Al-4V (Corail, Landos, France) provided with a bi-articulated femoral head (BHP, Zimmer, Swindon, UK). The coating thickness was 150 μm ± 50 μm; the purity and the crystallinity of the powder lots and coatings were checked by X-ray diffraction and infra-red spectrometry (Table 2).

The retrieved femurs were processed as previously described [16]. Briefly, they were fixed in a 4% formaldehyde solution in phosphate buffer. They were cut transversely into four or five parts and dehydrated in increasing ethanol solutions. They were embedded in polymethylmethacrylate (PMMA) and 2 mm thick sections were cut with a low speed cooled diamond saw. They were then polished down to a 50 μm thickness. They were surface stained with a Fucsin-Toluidine solution after they were etched for 2 minutes in 2% formic acid solution and 2 hours in a 20% methanol solution. Two sections each were prepared from the upper (metaphyseal), middle (diaphyseal) and lower (tip) third of the prosthesis. Other sections were made in regions of special interest. Sections were observed under either transmission or reflected light microscope (Reichert, Polyvar).

Some sections were stained with silver methenamine as described before [15]. Briefly, a 1% silver methenamine solution (pH 9.6) was deposited on sections for 1 minute at 2450 MHz in a microwave oven at 100 W. The staining solution was then reduced with a thio-carbohydrazide (TCH; Sigma, St. Louis, MO, USA) solution for 5 minutes and the sections were observed under a light microscope (as above). Also, after applying a carbon coating, the sections were studied, using the backscattering mode, in a scanning electron microscope (ISI-SS60) operated at an accelerating voltage of 25 kV. Energy-dispersive X-ray (EDX) microanalysis was performed on some sections using an EDAX-5000 (Philips, Mahwah, NJ) system equipped with a light element detector.

The surface of the middle third of two specimens was also examined using scanning electron microscopy (SEM). After 1 cm thick sections were cut from two fixed specimens implanted for three weeks and one year, the section was sawed longitudinally in two parts across the stem using a low speed cooled diamond saw. Then, one part of the implant was manually stripped of the femur endosteum, dehydrated with acetone, coated with gold palladium, and examined with SEM as mentioned in the previous paragraph. The other part of the specimen was immersed in a 12% NaOCl solution for five days (in order to remove the organic matrix), and processed identically for SEM.

The coating thickness after implantation was determined using image analysis (AES software, AES-Image, Toulouse, France). Serial measurements were made manually on some sections and the outside values were recorded. The percentage of the surface of the stem in contact with bone was obtained from the percentage of the stem perimeter in contact with bone. Manual measurements were made using the same software.
Bone response to HA-coated hip protheses

Figure 1. Bone morphotype found in contact with the metaphyseal coating in a one year implanted prosthesis. It is made of long trabeculae (arrow) having a direction perpendicular to the coating (HA). Reflected light microscopy after Von Kossa staining. Bar = 500 µm.

Table 3. Percentage of the coating surface in contact with bone (from four prostheses implanted for more than six months: 36, 20, 24 and 9.5 months).

<table>
<thead>
<tr>
<th>section level</th>
<th>percentage of coating in contact with bone (mean ± standard deviation, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>metaphysis</td>
<td>17.9 ± 9.04</td>
</tr>
<tr>
<td>diaphysis</td>
<td>51.13 ± 25.7</td>
</tr>
<tr>
<td>tip</td>
<td>82.73 ± 3.3</td>
</tr>
</tbody>
</table>

Table 4. Different bone morphotypes in contact with the HA-coating that can be individualized.

<table>
<thead>
<tr>
<th>Bone morphotype characteristics</th>
<th>orientation</th>
<th>location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 Long trabeculae bridging the endosteum to the prosthesis coating with no cross-links between the different trabeculae.</td>
<td>Perpendicular to the coating</td>
<td>internal and external prosthesis faces at the metaphyseal level</td>
</tr>
<tr>
<td>Type 2 Short trabeculae bridging the endosteum to the prosthesis coating with numerous cross-linking trabeculae.</td>
<td>Perpendicular to the coating</td>
<td>Internal and external prosthesis faces at the metaphyseal level. Face close to the endosteum at the tip level.</td>
</tr>
<tr>
<td>Type 3 Long and thin trabeculae making bone layers covering the coating</td>
<td>Parallel to the coating surface going in a transversal or longitudinal direction</td>
<td>Anterior and posterior prosthesis faces at the diaphyseal level.</td>
</tr>
</tbody>
</table>

Results

All the HA-coatings, except the five-day implanted one, were in contact with bone and bone marrow tissue. On sections from prostheses implanted for more than 3 months, different morphotypes could be observed bridging the prostheses surface to the femur endosteum. The morphotype of bone tissue at the coating contact differed greatly depending on the level from where the sections were obtained.

At the metaphyseal level, the percentage of the stem section perimeter in contact with bone is low (Table 3). This bone is made of long trabeculae, most of them having a direction perpendicular to the coating (Fig. 1 and Table 4). These trabeculae were inserted on the coating located on both the internal and external faces of the prostheses. The anterior and posterior faces did not show any bone insertion in most of the cases. Between bone trabeculae, a bone marrow normal cytology was present. Longitudinal sections prepared at the level of the junction between the coated and the uncoated regions of the neck, on a one year implanted prosthesis, showed that the uncoated region was surrounded by connective tissue and the coated region was surrounded by bone (Fig. 2). The transition zone between the fibrous tissue and the bone exactly corresponded to the junction between the metal and the HA-coating. Signs of osteoclastic resorption were evidenced on some sections from calcar regions. The histology of the calcar region of a one year implanted prosthesis, having received an allogeneic bone graft between the prosthesis and the cortex, showed that the graft had been osseointegrated. No dead bone with empty osteocyte lacunae was found.

The sections from the diaphysis showed more bone in contact with the coating (Table 3). In the upper middle part, a trabecular bone bridged the endosteal tissue to the internal and the external prosthesis faces (Fig. 3 and Table 4). The anterior and posterior faces of the stem showed very long trabeculae adsorbed along the bottom of the longitudinal grooves on the prosthesis surface (Fig. 4). In the lower middle part of the prosthesis, a dense trabecular bone tissue was in contact with the coating of the external and internal face (Fig. 5). Transversal bone layers were very often adsorbed on the coating on the anterior and posterior faces creating a new bone marrow cavity between this layer and the
Figure 2. Junction zone between the coated stem and the uncoated neck (stem alloy was removed from the section). Bone trabeculae (BT) were formed on the coating (HA) and there is a fibrous tissue (FT) opposite the uncoated neck. Bone apposition is stopped at the coating end (arrow). Reflected light microscopy of a nine months implanted prosthesis. Bar = 300 µm.

Figure 3. Trabecular bone morphotype present at the coating contact (HA) on internal zone of the diaphyseal region of a one year implanted prosthesis. Reflected light microscopy. Bar = 500 µm.

Note: Figures 4 and 5 are on page 130; the color plate on the facing page 129 has Figures 6 and 7.

Figure 6A. Low magnification of the diaphyseal part of a one year implanted prosthesis section. It shows a transversal bone layer (arrow) formed at prosthesis contact. This layer creates a bone marrow cavity (BMC) between itself and the endosteum. Some trabeculae (t) are bridging this layer to the endosteum. Fuchsin-Toluidine. Bar = 500 µm.

Figure 6B. Transversal bone layer on the coating (HA) of a nine month implanted prosthesis. The staining properties of the newly formed bone (NF) in contact with the coating shows that there is a remodeling process occurring in this zone. Numerous cement-line (arrowhead) appearing like arrest-line can be identified between the bone region formed at different times. (OB = old bone). Toluidine blue. Bar = 150 µm.

Figure 7A. Immature bone (IM) bridging the endosteum to a 36 month implanted prosthesis. Bone still displays an immature structure with no distinguishable osteon suggesting that this is remodeling zone. Resorption cavity (zone of several former osteon resorption) (RC) and osteon remodeling (arrow) are visible in the cortical bone. Fuchsin-Toluidine. HA: hydroxyapatite coating. Bar = 250 µm. Figure 7B. Microtome section of the bone in contact with a nine-month implanted prosthesis. There is a clear delimitation (arrow) between the old cortical and mature bone (M) and the newly formed cancellous and immature (IM) bone bridging the prosthesis coating (P), which has been removed from the section to the endosteum. The newly formed bone is immature with no collagen layer and containing numerous osteocytes. Goldner trichrome. Bar = 300 µm.
Bone response to HA-coated hip protheses
Figure 4 (above). Long bone trabeculae (BT) formed at the bottom of some longitudinal grooves found on the prostheses surface at the diaphyseal level. Reflected light microscopy of a one year-implanted prosthesis after NaOCl treatment. Bar = 500 μm.

Figure 5 (above). Dense trabecular bone found at the coating (HA) contact in the low diaphyseal region of a three year implanted prosthesis. Fuchsine-Toluidine. Bar = 100 μm.

See page 131 for legends for Figures 8, 9, 15, and 16.
Bone response to HA-coated hip protheses

**Figure 8.** As soon as five days after the surgery, fibroblastic cell proliferation (arrowhead) occurred around the bone trabeculae (T) located in close proximity to the coating (HA). Some of these cells are at this time very close to the coating surface. Fuchsin-Toluidine. Bar = 200 μm.

**Figure 9.** Osteoid (OS) formation at the coating (HA) contact one month after implantation by osteoblasts (arrow) opposed on the ceramic. Fuchsin-Toluidine. Bar = 15 μm.

**Note:** Figures 10-14 are on the following pages.

**Figure 15.** Lining cells (arrow) present on the surface of bone formed on the ceramic 1.5 months after implantation. An osteoid matrix (OM) is interposed between the cells and the coating (HA). Fuchsin-Toluidine. Bar = 50 μm.

**Figure 16.** Light micrograph of the bone marrow tissue at the contact of the HA-coating (HA) of a one year implanted prosthesis. The bone marrow tissue found at the ceramic contact is made of erythroblasts (E), normoblasts (N); myeloblasts (M) and promyelocytes. A few multinuclear cells (MN) may also be found. Fuchsin-toluidine Bar = 50 μm.

endosteum (Fig. 6). One year after implantation, the newly formed bone was distinguishable from the old bone due to different staining properties even after maturation. Some small particles of old bone were dispersed in the newly formed matrix; this suggests an inclusion of bone fragments created by reaming in the new bone, bridging the gap between the endosteum and the prosthesis.

The bone found in the tip regions was very polymorphous. When located close to the endosteum, the tip was in contact with trabecular bone; in the places far away from endosteum, the tip touched the thin transversal bone layer (Fig. 7). Longitudinal sections showed that trabecular bone was formed in the bone marrow cavity caudal to the tip. It should be noted that three years after implantation, the zone between the endosteum and the prosthesis tip was still a highly remodeling zone. Immature bone was still present and some marks of osteon remodeling were visible in the internal cortex; many of resorption cavities and reversal lines were found.

In the early stages of bone formation (less than 4 months), the different morphotypes were not discernible; immature trabecular bone was occupying most of the space between the endosteum and the ceramic coating.

Several stages of bone formation could be distinguished. Five days after implantation, bone fragments made during the reaming were seen at the coating contact. Fibroblast-like proliferation occurred around the fragments. Some of these cells migrated from fragments to the coating (Fig. 8). SEM showed that proteins and fibroblast-like cells were deposited on the coating.

By three weeks to one month after the implantation, the hematoma found around the prosthesis in the early implantation time was partially resorbed and a highly vascularized loose connective tissue had invaded the bone marrow cavity around the stem. Some osteoblasts, differentiating from the fibroblast-like cells contained in the loose connective tissue according to a direct bone formation process, synthesized some osteoid tissue under a trabecular form in the entire volume of the bone marrow cavity (Fig. 9). No zone of endochondral bone formation was evident.

Two months after implantation, light microscopy showed that osteoblasts were at the HA-coating surface contact and synthesized osteoid by the cell pole facing the coating. Thus, bone extracellular matrix (ECM) was laid down on the coating. After the organic phase removal, SEM showed that mineralization of the bone ECM had occurred. Some calcium phosphate deposits were found on the coating and identified by EDX. These deposits were randomly assembled and formed a very immature bone. This mineralized matrix was partly in contact with the coating- bridging and making arches over some coating irregularities (Fig. 10).

By six to nine months, the consequences of a remodeling process were apparent on the bone in the close vicinity of the prosthesis. The immature bone was
The grains of the coating surface once the amorphous phase was removed, were available for phagocytosis by mononuclear or multinuclear cells. On this section, a multinuclear cell (arrowhead), close to a bone trabeculae (T) is apposed on the HA-coating (HA) and is phagocytosing an HA-grains (arrow). M: Bone marrow cavity. Silver methenamine. Bar = 20 μm.

Figure 11B. EDX spectrum of the particle found (for a live time of 166 seconds) in the multinuclear cell in Figure 11A.

progressively replaced by a mature tissue showing a layered structure. At this period, light microscopy showed some HA-grains phagocytosed by some multinuclear or mononuclear cells in contact with the coating (Fig. 11). SEM showed that the remodeling process affected also the coating ceramic. A very rough coating, having a different appearance from the smooth aspect visible immediately after the implantation, was evidenced. The coating’s amorphous phase had been removed by cells or had been dissolved in the extracellular fluids, making the coating grains apparent (Fig. 12).

From this stage, the coating remodeling was clearly visible. Some multinucleated or mononucleated cells contained calcium phosphate particles. These cells were located either close to the coating or at a few hundred micrometers from it. The majority of the particle-containing cells were located at the coating contact. All the prostheses did not show the same resorption rate of their coating (Table 5). One of them, which had been implanted for two years, had been nearly totally resorbed, although another two-year implanted prosthesis showed very little signs of coating degradation. However, resorption of calcium phosphate coatings was followed by bone ingrowth inside the resorbed region as shown.
by the engulfment of coating debris inside the newly formed bone. Bone trabeculae were sometimes found at the contact of the sand-blasted alloy surface in sections coming from the two-year degraded implant. Degradation marks were stronger in regions facing the bone marrow cavity than in regions where bone was inserted (Fig. 13). The alteration of the coating thickness was not strictly correlated to the implantation time (Table 5). Based on an examination of the sections coming from the 36 months implanted prosthesis, the resorption was greater in the metaphyseal region. One prosthesis implanted for 1.5 months showed a mechanical alteration of the coating. Small fractions of the ceramic, located at the top of diaphyseal ridges, were detached from the metal and were included inside bone trabeculae which were formed at the metal and ceramic contact (Fig. 14).

Forming or remodeling bone is not the only kind of bone tissue shown at the ceramic contact. Resting bone

Table 5. Extreme values of the coating thickness (µm) of prostheses implanted for various periods. Control material is the thickness values measured on a unimplanted prosthesis.

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>1.5 months</th>
<th>7 months</th>
<th>12 months</th>
<th>24 months</th>
<th>43 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metaphysis</td>
<td>110-153</td>
<td>104-143</td>
<td>137-163</td>
<td>99-220</td>
<td>0-183</td>
<td>58-164</td>
</tr>
<tr>
<td>Middle diaphysis</td>
<td>101-147</td>
<td>123-182</td>
<td>102-170</td>
<td>50-164</td>
<td>0-180</td>
<td>104-204</td>
</tr>
<tr>
<td>Tip</td>
<td>99-185</td>
<td>119-164</td>
<td>155-213</td>
<td>40-184</td>
<td>0-188</td>
<td>119-163</td>
</tr>
</tbody>
</table>

Figure 14. Reflected light micrograph of a section of a 1.5 month implanted prosthesis showing fragments of coating (*) located at the top of surface ridges, detached from the alloy probably during prosthesis insertion. These fragments were included inside newly formed bone (arrow). Bar = 100 µm.
trabeculae was limited by lining cells [Fig. 15 (see page 130, color plate)] and can be shown on the coating surface.

Bone marrow tissue is found at the contact of the ceramic coating of all prostheses once new bone tissue was formed. The cytology of this tissue close to the coating is made of erythroblasts, normoblasts, myeloblasts, and promyelocytes. Very few macrophages or multinucleated cells were adsorbed on the ceramic during the first months of implantation. Their number increased when the coating had begun to degrade and when ceramic grains were released {(Fig. 16 (see page 130, color plate)}.

Discussion

This study confirms the experimental results obtained on animals and clinical results found in humans. Several authors have already showed that osseointegration of HA-coated hip stems was obtained in humans [9, 16, 18, 22, 25, 33, 37]. However, our series consisted of fifteen successful prostheses implanted for various periods, one implanted three years. It allowed us to follow the behaviour of successful materials during a medium term implantation period.

Osseointegration of the bioactive HA-coatings was shown in all cases. At the light microscope level, no fibrous tissue interposition was evident. In vitro, de Bruijn et al. [10] and Orr et al. [36] demonstrated that a cement line-like layer was present between the newly formed bone and the ceramic. The interfacial structure may change according to the coating characteristics and particularly the crystallinity [11, 19].

Bone formation is a direct ossification process resulting from the healing reaction occurring in the bone marrow after a lesion. We could not see the physico-chemical surface modifications described by de Bruijn et al. [10] or by Bagambisa et al. [2] on the coating surface. The short term implanted anorganic specimens (less than two months), however, had calcium phosphate deposits formed on the ceramic coating. These calcium phosphates had presumably nucleated and grown inside the osteoid matrix. The mineral phase which occurred at the coating contact during short term implantation was very heterogeneous. Some coating sites were in contact with a well mineralized immature bone, while other sites showed a few deposits formed on the coating. As bone was in contact with coating at a few points, it is suggested that most of the bone mineral phase formed in the coating proximity is not in continuity with the epitaxial crystals grown on the surface [7].

Several bone morphotypes were found depending on the level from which sections were selected. The trabecular morphotype found in the metaphyseal region could be attributed to the natural structure of the cancellous bone found at this level. However, the low percentage of the ceramic surface in contact with bone in this region suggests that a stress shielding process occurred at that location. The evolution of the bone morphotype is another argument for a remodeling process due to the stress modification induced by the implant. The development of cancellous immature bone in most of the bone marrow volume and the persistence of mature bone at the contact of the same regions for all the prostheses suggest that the bone stays only in highly stressed regions.

The percentage of the implant surface at the bone contact is higher in the tip or diaphyseal region of prostheses suggesting that these regions are subjected to a higher stress than the metaphyseal zone.

Calcar and trochanter sections have shown that the bone appeared in contact with the implant only if the coating was applied onto the metal. The naked titanium alloy was in contact with dense fibrous tissue indicating that the bioactive coating is necessary for bone apposition on the prosthesis. No migration of polyethylene debris was seen lower than the zone of bone apposition at the beginning of the coating. Osteoclastic resorption of the calcar could be detected by X-ray radiography.

Cell resorption of HA-ceramic coating once implanted in humans has been described by several authors [6, 18]. This degradation was attributed to the low pH extracellular compartment induced by osteoclasts. All calcium phosphate phases show a high solubility in this range of pH [5]. Furthermore, the coating characteristics have an influence on the rate of the ceramic degradation [19, 30, 32]. The percentage of porosity and the amount of amorphous phase were shown to increase the coating degradation. The roughness of the sand-blasted surface on which the ceramic is mechanically linked or the geometry of the macrostructure existing on the prosthesis may favor a delamination. The small fragments detached from the coating implanted for 1.5 months were located at ridge tops and their detachment was probably due to the shear stress existing at this location during the implantation, creating zones of fragility. It is important to note that coating resorption is not only a degradation process. The inclusion of coating fragments in bone trabeculae and the apposition of bone trabeculae on the alloy in some resorption areas indicate that a phase of bone formation follows the resorption states. The phagocytosis of ceramic particles by bone marrow cells is evident in close proximity to the coating only, probably because the degradation of grains in the low pH cell compartments takes place before the cells migrate.

Complications due to the debris emission by the coating have been described: migration of calcium phos-
Bone response to HA-coated hip prostheses

Phosphate particles in the joint increasing the polyethylene degradation by a third body wear mechanism and osteolysis. We have also shown the presence of calcium phosphate crystals at the polyethylene cup surface, however, no sign of localized osteolysis around the bone marrow regions containing debris or in proximity to coating degradation areas was noted [20]. Moreover, ceramic debris were very often included inside bone trabeculae without any fibrous or membranous interposition making the behaviour of bone tissue in the presence of HA-ceramic debris very different from its behaviour on contact with polyethylene or metal debris. This difference between HA and metal debris has been already demonstrated experimentally in the animal [24]. Furthermore, these results are consistent with the synthetic HA-particles bone incorporation described by Wang [40]. However, HA crystals have been shown to be inducers of cytokines release [1, 38]. The physicochemical differences between calcium phosphate crystals found in chondrocalcinosis process [21] and the synthetic HA-particles released by coatings can explain the difference of cell and tissue reaction at their contact.

The mechanism of HA ceramic resorption has been reported to be both a cell and solution mediated mechanism [19, 23, 39]. We found most of the small size debris (< 50-60 μm) phagocytosed by mononuclear or multinuclear cells. These cells were also present at the coating contact. Scanning electron microscopic examination of anorganic specimens showed that the amorphous phase of prostheses implanted for more than one year was initially resorbed. The resorption homogeneity of the amorphous phase suggests that it is mostly a solubilization by extracellular fluids. The solubilization and cell degradation of the amorphous phase increase the coating microporosity and make the coating grains available for cell phagocytosis.

HA-coatings used as thin layers on alloys are produced by a plasma spray process. HA powder is introduced in a high temperature plasma gas obtained between two electrodes. The powder grains, which are partially melted, are then ejected toward the sand-blasted surface of the alloy. The partially liquified grains adapt to the shape of the surface irregularities and, on cooling, form a thin layer of HA-ceramic on the surface [31]. This plasma-spray process makes it possible to obtain calcium phosphate ceramic coating with very different characteristics depending on plasma-spray parameters and powder characteristics. In our series, coating characteristics were identical for all implants and no conclusions could be drawn on the influence of the coating characteristics on this type of coating’s osseointegration in humans. It should be noted that this extent of coating crystallinity, thickness, roughness, and crystal structure is compatible with osseointegration in humans.

Conclusions

HA-coated prostheses, once implanted in humans, are osseointegrated. Several bone morphotypes were formed in contact with the coatings. The formation was governed either by stress acting on the newly formed bone or by the structure of bone in which the newly formed bone appeared or by both mechanisms. The osseointegration process of calcium phosphate coatings implies a remodeling of this ceramic material. During the remodeling process, calcium phosphate particles were released, phagocytosed and solubilized or osseointegrated. Despite the presence of particles inside bone tissues, no osteolysis was evident in the vicinity of this material at the predominantly short time periods in this series of implants.

References


Bone response to HA-coated hip protheses

metaphyseal section
diaphyseal section
tip section

Figure 17. Location of prosthesis sections used for image analysis.


Discussion with Reviewers

O. Johari: Can you please provide a sketch showing the location of various sections on the prosthesis.

Authors: Please see Figure 17.

U. Gross: What is the reason for the resorption of HA?

Authors: HA-coatings, with characteristics such as those described in the text, are composed of an amorphous phase and a crystalline phase. The amorphous phase is more soluble than the crystalline one and may contain calcium phosphates other than HA (α and β-TCP, and tetracalcium phosphate) and CaO. Some of these phases are known to be more soluble than HA. Therefore, the amorphous phase can be both solubilized in the extracellular liquid and solubilized in the resorption chamber of osteoclasts, in which the pH is low. Once the amorphous phase is resorbed, the ceramic grains constituting the coating crystalline phase are released and phagocytosed by macrophages, in which they are solubilized in low pH cell compartments (lysosomes).

U. Gross: Did you find reduced density of the hydroxyapatite coating in areas of resorption?

Authors: The density of HA-coating once implanted was not measured. However, we could see, by SEM, an increased coating porosity due to the dissolution of the amorphous phase of the HA-coating.

U. Gross: Why did you find osteoid at the surface of the hydroxyapatite coating (Fig. 15)? Did the patient suffer from a disease concerning mineral metabolism?

Authors: To our knowledge, the patient did not suffer from a disease concerning mineral metabolism. Osteoid tissue is very often found at the contact of HA-coating. Although no morphometric measurements concerning the amount of osteoid were made, the osteoid tissue found at the coating contact did not evoke mineralization problems such as osteomalacia; rather, it caused an increased remodeling process, as shown by the coating resorption and bone ingrowth. It should be noted that no abnormality of matrix mineralization was seen by backscattered SEM at the coating contact.
J.D. de Bruijn: In several sections in the manuscript, the authors mention the presence of calcium phosphate crystals on the coating. Were these crystals, or deposits, associated with collagen fibers? I ask this question since we (in collaborative projects with Prof. J.E. Davies, Centre for Biomaterials, University of Toronto) have previously shown and published that, both in vitro and in vivo, differentiating osteoblasts produce afibrillar globular calcified deposits, prior to collagen fiber incorporation. In fact, could the structure shown in Figure 12 partially represent the above mentioned afibrillar biological mineralized deposit?

Authors: As the calcium phosphate deposits observed at the coating surface were shown after organic matrix removal, it is very difficult to know what the links and the relations of these structures are with collagen. Moreover, these deposits have various sizes and most of them are much larger than the globular afibrillar deposits described by de Bruijn et al. [11]. Furthermore, they are very often close to organized structures evoking suggestions of immature bone.

J.D. de Bruijn: In Table 5, the authors mention bone and coating remodeling in stage 3. This, together with several statements in the text, indicates that the coating can participate in the process of bone turnover and can thus be resorbed by osteoclasts. What proof do the authors have that the HA-coating can indeed be resorbed by osteoclasts (via the excretion of protons and enzymes in the extracellular microenvironment produced by these cells), besides showing that multinucleate cells phagocytose small grains detached from the coating surface?

Authors: Several publications have shown that osteoclasts in vitro and in vivo can resorb HA-ceramics. We have shown that, in vivo, when osteoclasts were apposed at the ceramic surface, there was a decrease of the ceramic density below the cell. As the major difference between HA-ceramic coatings and regular HA-ceramics is the presence of an amorphous phase, we considered that some multinucleated cells found at the bottom of resorption cavities in the coating were osteoclasts which had resorbed the ceramic coating.

Reviewer VI: Since EDX was used in this study and considering that the crystallinity of the HA-coating was on the range of 60%, have the authors looked in the predominant phase of the debris found in resorbed areas of the coating? Would the resorption occur primarily in the amorphous phase of the coating?

Authors: As seen in SEM, the amorphous phase is primarily resorbed, thus making the grains of the ceramic apparent. According to the debris shape and to the morphology of grains appearing on the coating, once the amorphous phase is resorbed, it seems that the predomi­nant phase within the cells is the crystalline phase. However, we could not determine precisely by EDX the proportion of both phases in the cells.

Reviewer VI: What was the geometry of the Ti-alloy surface to which the HA-coating was applied?

Authors: The surface was sandblasted to obtain a roughness compatible with HA-ceramic anchoring.