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Biocompatibility of hydroxyapatite-coated hip prostheses*

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Summary. In three patients a mechanically well-fixed Mathys Ceros 80 (Ha) hydroxyapatite-coated acetabular component was revised 2, 5 and 13 months after total hip replacement due to component malposition. In each case there was a thin cellular connective tissue membrane between hydroxyapatite and bone, the main cell type being fibroblast with only occasional giant cells. Immunohistological analysis revealed some MHC locus II antigen positive cells that were identified as monocytes. No interleukin-2 receptor positive cells were found. Under clinical cyclic loading conditions there does not seem to be chemical fixation or bony ingrowth into the hydroxyapatite coated prosthesis component. In human lymphocyte cultures, hydroxyapatite (Interpore 200, particle diameters 15–40 μm) did not cause an increase in lymphocyte DNA synthesis as assessed by the ^{3}H-thymidine incorporation method on culture days 1, 3 and 5. As analysed with lymphocyte activation markers, the hydroxyapatite-dependent expression of MHC locus II antigen was modest and differed significantly ($P < 0.05$) from that in culture medium only on day 3. Hydroxyapatite induced only a slight interleukin-2 receptor expression that did not differ from culture medium on days 1, 3 and 5. CD4 and CD8 positive lymphocytes as well as monocytes were not seen attached to hydroxyapatite particles during the culture days. Our findings suggest that hydroxyapatite is an immunologically inert implant material.

The belief that technically well-inserted total hip prostheses will cause little or no adverse tissue reaction is still common [5]. Harris and associates [8] were the first to report aggressive granulomatous-type tissue reactions against well-inserted cemented total hip prostheses and this phenomenon is commonly called the "cement disease" [13]. Recent research has suggested a foreign-body-type reaction in aggressive granulomatosis with an uncoupling of the normal sequence of monocyte-macrophage-mediated clearance of foreign material and fibroblast-mediated remodelling of the extracellular matrix [20]. The current trend has been to use more cementless total hip prostheses to avoid adverse tissue reactions, but aggressive granulomatous lesions are also seen in cementless total hip replacements [21, 22]. Hydroxyapatite has proved to be a very promising orthopaedic biomaterial, and when it is used as a bone graft substitute in man or experimental animals, bone ingrowth into the implants and an absence of adverse reactions have been demonstrated [1, 10]. On the basis of this knowledge, manufacturing of hydroxyapatite-covered prosthetic components has been a logical means to improve the incorporation of the prosthesis into the bone without causing adverse foreign-body-type reaction. Hydroxyapatite-based total hip prosthesis components are relatively widely used today. We evaluated biocompatibility of hydroxyapatite in vivo and in vitro.

Materials and methods

Histology and immunohistology

In three patients a mechanically well-fixed Mathys Ceros 80 (Ha) hydroxyapatite-coated acetabular component had to be revised 2, 5 and 13 months respectively after the total hip arthroplasty due to component malposition. Patient 1 was a 65-year-old woman who had suffered from seronegative rheumatic arthritis (erythrocyte sedimentation rate 60) for 12 years. Her hydroxyapatite-coated acetabular component had to be revised 2 months postoperatively, since there was a tendency for luxation due to excess retroversion of the acetabular component. The cup was well fixed, but as yet there was macroscopically no bony ingrowth into the hydroxyapatite and the adhesion between the acetabular component and bone was not very strong. Patient 2 was a 41-year-old woman with dysplastic hip. In her case too there was recurrent luxation due to malposition of the acetabular cup and this was revised 5 months after the primary arthroplasty. Macroscopically there was a very thin membranous layer and strong adhesion between the acetabular

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component and bone. Patient 3 was a 66-year-old man with osteoarthrosis of the hip whose clinically well-fixed prosthesis had to be revised 13 months postoperatively because the original prosthetic components were too small and caused mechanical dysfunction. At the revision operation, the acetabular component was found to be well-fixed and there was strong adhesion between the acetabular component but no evidence of bony ingrowth. There were no signs of infection in any of the three revised hips, the bacterial cultures were negative, and except in patient 1 the erythrocyte sedimentation rates were normal. In all three patients, samples of interface tissue from between the acetabular component and bone were analysed by routine microcscopy using haematoxylin-eosin staining, and one patient's sample (patient 1) was also analysed for T-cell subsets (immunohistological stainings) according to our laboratory routines [20].

Human lymphocyte cultures

Fine pulverised sterile Interpore 200 hydroxyapatite (particle diameters 15–40 μm) was analysed in human lymphocyte cultures [19]. Venous blood was obtained from five healthy volunteers in syringes containing preservative-free heparin. After dilution with RPMI-1640 (1:1) a Lymphorep (specific gravity 1.078 μg/ml, Nyegaard, Oslo, Norway) density gradient isolation and peripheral blood mononuclear cells was performed (400 × g, 40 min, +22°C). 2×10^5 isolated and washed cells were placed in 0.2-ml (Falcon 3072, Becton Dickinson, Oxnard, Calif.) or 2.0-ml (Nucleon Delta SI, Roskilde, Denmark) flat-bottomed cell culture wells. In a preliminary study 10-μg, 100-μg, 1000-μg and 10000 μg quantities of the above described fine pulverised hydroxyapatite were tested. One milligram per well (2 ml) was used in subsequent experiments, because hydroxypaptite had shown no toxic effect and 1 mg could easily be visualized as microscopic particles in the cytocentrifuge specimens. As negative controls we used plain 10% FCS-RPMI-1640 media without hydroxyapatite and as positive control phytohaemagglutinin (PHA, 1.25 μg per well; Wellcome Diagnostics, Dartford, UK). The lymphocyte culture was kept in a 5% CO_2-air mixture at 37°C. End-point analysis was performed 0, 1, 3 and 5 days later for analysis of the activation kinetics [14].

Avidin-biotin-peroxidase (ABC) staining

The cultured cells were pipetted and cytocentrifuge preparations were made on days 0, 1, 3 and 5. The avidin-biotin-peroxidase complex (ABC) staining sequence was performed according to Hsu et al. [11]. The following monoclonal phenotype and activation marker antibodies were used: CD4 (helper-inducer T cells) and CD8 (suppressor-cytotoxic T-cells), DAKOT4, DAKOT8, Dacopatts, Copenhagen, Denmark); MCH locus II antigen (activated lymphocytes, monocytes), (OKIa, Ortho Pharmaceuticals Co, Raritan, NJ); interleukin-2 receptor (activated T cells), [OKT26a (CD25), Ortho Pharmaceuticals Co, Raritan, NJ]; C3bi- receptor (monocytes and granulocytes; OKM1, Ortho Pharmaceuticals Co., Raritan, NJ).

^{3}H-Thymidine incorporation

Parallel to the ABC-staining sequence, 1 μCi (6-^{3}H-thymidine (specific activity 5 Ci/mmol, Amersham, UK) was added to each well 18–24 h before the cultures were harvested with a semi-automatic multi-sample harvester (MHCI, Skatron, Lierbyen, Norway) on glass-fibre filters in triplicate on days 0, 1, 3 and 5. The filters were suspended in a scintillation solution before β-scintillation counting in CK B Wallac 81000 counter (Wallac, Turku, Finland).

Standard error of the mean was used to express dispersion and the significance of differences between the mean values was determined with the Mann-Whitney U test.

Results

Histology and immunohistology

In patient 1, in the undecalcified microscopic section of the hydroxyapatite/bone interface tissue, fibroblasts and many foreign body giant cells were visible, and some of them contained hydroxyapatite particles. Centrally, there was a very cellular connective tissue loosely arranged. Toward the periphery, the loose connective tissue was replaced by denser connective tissue, which was not of osteoid type.

In patients 2 and 3, light microscopy revealed very cellular connective tissue, in part loosely arranged and with some strands of more dense connective tissue, and

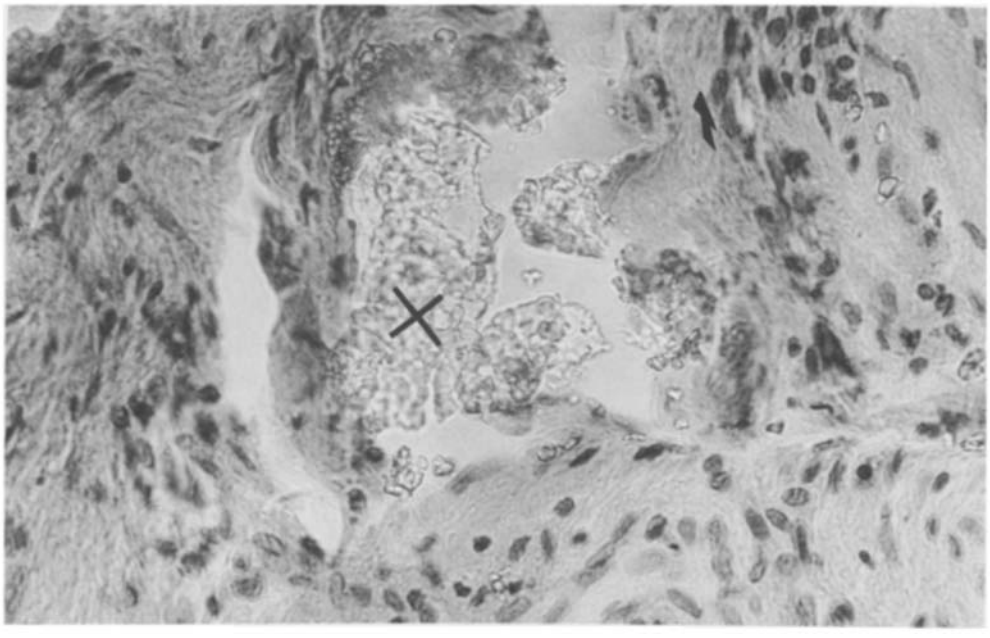

Fig. 1. Histological slide with haematoxylin-eosin staining of the interface membrane between hydroxyapatite-coated acetabular component and bone at 5 months after arthroplasty (patient 2). There was good component fixation. Cellular connective tissue with a number of fibroblasts and occasional giant cells were seen. *X*, Hydroxyapatite particle. Original magnification × 300

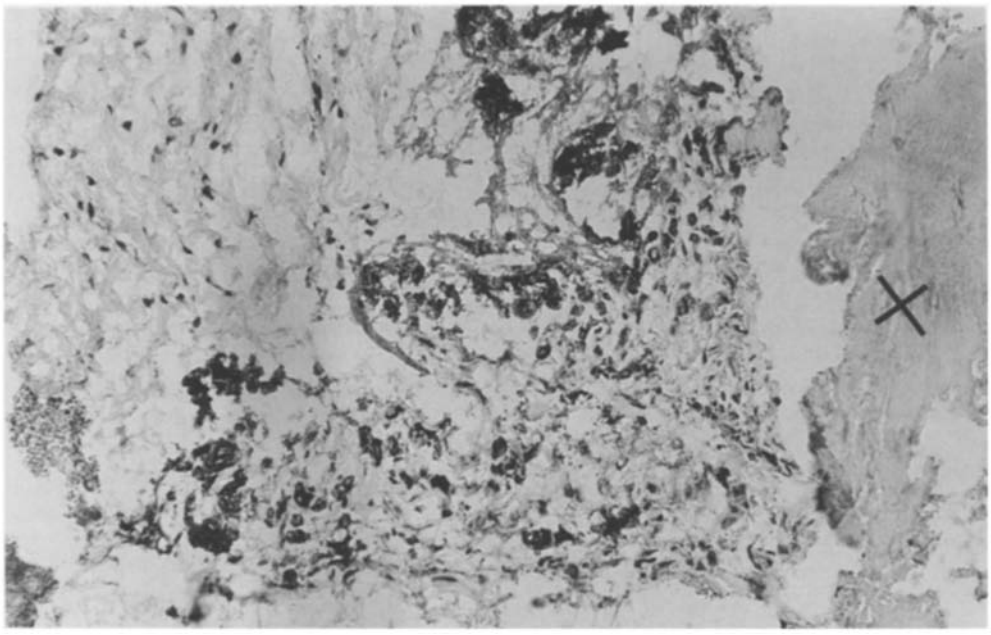

Fig. 2. Immunohistological analysis of the interface membrane between hydroxyapatite-coated acetabular component and bone at 2 months (patient 1). There were C3-bi positive cells in the dense connective tissue area. On the right a particle with cartilage-like appearance (*X*). Original magnification × 300

Table 1. Hydroxyapatite- (1.0 mg/2 ml) and PHA- (phytohaemagglutinin 1.25 μg/ml) induced proliferation in peripheral blood mononuclear cell cultures as compared to negative controls (culture medium alone). Mean ± SEM

Experimental set-up	Proliferation (cpm/0.2-ml well) at culture day			
	0	1	3	5
Culture medium alone	249.4 ± 10.0	1294.8 ± 781.7	1129.6 ± 310.6	1515.0 ± 510.3
PHA	229.4 ± 10.2	18241.2 ± 5702.6	45362.2 ± 2249.3	6928.0 ± 844.1
Hydroxyapatite	250.2 ± 6.8	1195.0 ± 508.1	1196.8 ± 338.0	1757.4 ± 487.1

* Days 1–5, $P < 0.05$
** Days 0–5, $P > 0.05$
Mann-Whitney *U* test

there were occasional giant cells (Fig. 1). Some hydroxyapatite was seen as occasional islands in the middle of the connective tissue. All three revised acetabular components were considered to be very well fixed. However, we did not find direct bony ingrowth into hydroxyapatite; in each case we found a thin connective tissue membrane between bone and hydroxyapatite.

Immunohistological findings of the sample from patient 1 revealed some MHC locus II antigen positive cells in the dense conncetive tissue area that were identified as monocytes, since C3-bi positive cells were also present in consecutive histological slides (Fig. 2). Virtually no interleukin-2 receptor (CD25) positive cells could be seen, and only occasional CD4 and CD8 positive lymphocytes were detected.

^{3}*H-Thymidine incorporation*

Hydroxyapatite did not cause an increase in lymphocyte DNA synthesis as assessed by the ^{3}H-thymidine incorporation method. The lymphocyte proliferation caused by PHA showed that the indicator cells had a full action capacity. Table 1 presents the hydroxyapatite-induced proliferation in peripheral mononuclear cell cultures compared to negative (culture medium alone) and positive mitogen (PHA 1.25 μg/ml) controls. The proliferation registered for hydroxyapatite did not differ from the one caused by culture medium alone. On days 1, 3 and 5, the proliferation in the PHA-dependent cultures was significantly ($P < 0.05$) higher than both the hydroxyapatite-dependent and the control mediums.

Lymphocyte activation markers

The PHA-driven lymphocyte culture showed a marked increase in the expression of both MHC locus II antigen and interleukin-2 receptors that significantly differed ($P < 0.05$) from both control cultures and the cultures containing hydroxyapatite on days 1, 3 and 5 (Table 2). The hydroxyapatite-dependent expression of MHC locus II antigen was modest and differed significantly ($P < 0.05$) from that in culture medium only on day 3 (Fig. 3). Hydroxyapatite induced only slight interleukin-2 receptor expression that did not differ from culture medium on days 1, 3 and 5.

CD4 and CD8 positive lymphocytes as well as monocytes were not seen attached to hydroxyapatite particles during the culture days.

Table 2. Lymphocyte activation profile in peripheral blood mononuclear cell cultures exposed to hydroxyapatite, negative (culture medium alone) and positive (phytohaemagglutinin, PHA) controls. Mean ± SEM

	MHC locus II antigen				Interleukin-2 receptor			
	0	1	3	5	0	1	3	5
Culture medium alone	5.7 ± 1.3	8.0 ± 2.3	12.1 ± 1.7*	19.9 ± 1.0	3.7 ± 0.8	5.5 ± 1.1	6.9 ± 1.0	14.5 ± 2.1
PHA	6.2 ± 1.4	24.7 ± 5.0	71.5 ± 3.6	47.8 ± 1.3	10.5 ± 2.3	29.6 ± 3.6	49.4 ± 1.8	67.0 ± 5.9
Hydroxyapatite	6.1 ± 0.6	13.0 ± 1.9	25.9 ± 2.8*	20.3 ± 4.0	10.3 ± 2.7	13.5 ± 2.7	10.9 ± 1.0	11.4 ± 2.5

* $P < 0.05$ Mann-Whitney U test

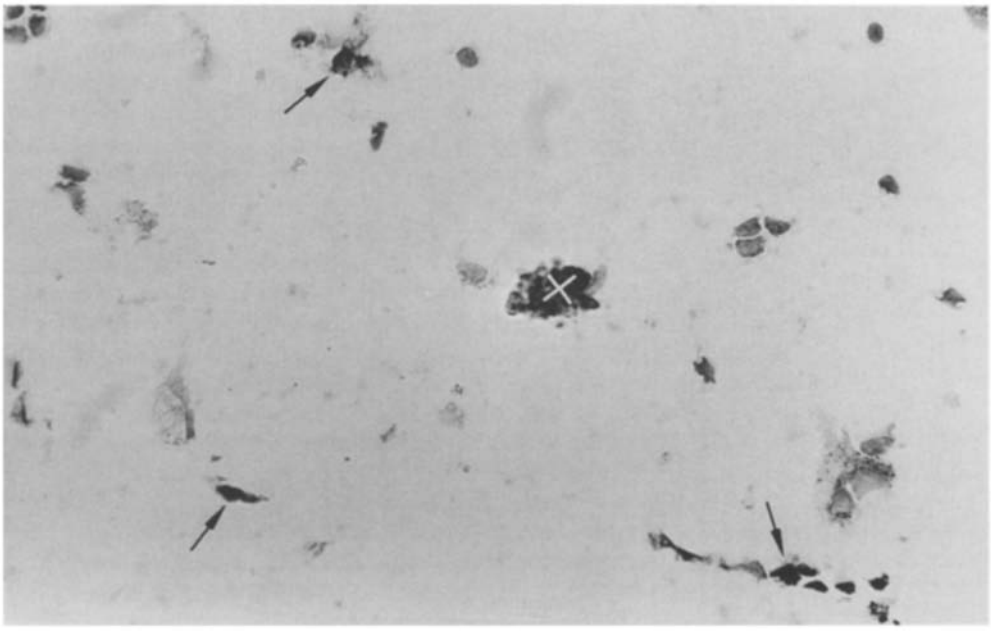

Fig. 3. Hydroxyapatite-dependent human lymphocyte culture stained with MHC locus II antigen. Hydroxyapatite particle (X) without mononuclear cell attachment. MHC locus II positive cells marked with *arrows*

Discussion

In the early period of total hip replacement surgery, it was thought that well-inserted prostheses with methylmethacrylate fixation would be practically inert and would not induce adverse tissue reaction [2, 3]. With time, it became obvious that there would be no bony ingrowth into the methylmethacrylate mantle around the prosthesis and a pseudosynovium-like membrane is generated as an interface between bone and cement [4, 15] and even in well-fixed prostheses this membrane includes macrophages and osteoclasts [16]. Recent research has pointed out immunobiological factors in often unpredictable adverse reactions to commonly used prosthetic implant materials [18, 20–22, 25]. Cementless total hip replacement has not proved to be a guarantee against adverse tissue reactions [21, 22]. Probably all new implant materials should undergo investigation concerning their immunobiological response.

In several studies evidence has accumulated indicating that ceramic forms of hydroxyapatite would be inert as implant material and do not induce adverse tissue reaction when implanted in bone [1, 10]. Hydroxyapatite has been suggested to be biocompatible, easily manufactured to any size or shape, and to offer a chemical environment and surface conducive to new bone formation [12]. Hydroxyapatite is a bioactive ceramic and is thought to bond to bone chemically, in contrast to all previously available implant materials [6, 9]. Stephenson et al. [24] have shown that the presence of hydroxyapatite induces bone formation to close even relatively large gaps. In the hope of achieving good fixation and avoiding adverse tissue reactions, hydroxyapatite-coated total hip prosthesis components have recently become increasingly popular. To date, data on the biocompatibility of hydroxyapatite-coated prosthesis components has been based on indirect evidence or animal experiments. Geesink [7] has reported excellent 2-year clinical and radiological results with hydroxyapatite-coated prostheses. Quite recently, Osborn [16] suggested on the basis of a human autopsy specimen that at 7 weeks after implantation there was bone ingrowth into the hydroxyapatite coating of a hip prosthesis.

Our three patients showed that clinically good fixation/adhesion can be achieved with a hydroxyapatite-coated acetabular component. However, there was no bony ingrowth into the hydroxyapatite, and in each case there was an interface membrane consisting of connective tissue which included occasional macrophages and virtually no cells of the lymphoid series. This speaks

against the theory that hydroxyapatite binds chemically to bone, at least under cyclic loading conditions. The tissue response to the implant was modest, with occasional signs of foreign body reaction. To date, there is no clinical evidence that any total hip implant material under clinical loading conditions is fixed without a connective tissue interface, and this is apparently also the case with hydroxyapatite.

The human lymphocyte culture method used here has been proved to be a reliable method for investigating immunobiological response to biomaterials [19, 23]. In human lymphocyte cultures, the ^{3}H-thymidine incorporation method appears to be relatively insensitive to slight changes in lymphocyte activation caused by hydroxyapatite. With the more sensitive activation marker method, the hydroxyapatite-dependent culture showed some MHC locus II expression on the surface on monocytes from day 1 onward. However, in hydroxyapatite-dependent lymphocyte cultures, the activated monocytes did not appear to induce subsequent lymphocyte activation; the number of MHC locus II positive cells on days 3 and 5 of hydroxyapatite stimulation was much lower than in PHA stimulation. In addition, only slight interleukin-2 receptor expression on the lymphocytes was seen on days 3 and 5 in the hydroxyapatite-dependent lymphocyte cultures. In contrast to previous observations with methylmethacrylate, no lymphocytes and monocytes were found attached to the hydroxyapatite particles [23].

The lymphocyte activation seen in peripheral blood mononuclear cell cultures exposed to hydroxyapatite suggests that there is a non-specific monocyte activation due to the presence of phagocytosable particles in the culture. This kind of non-immunological monocyte activation may also be responsible for the cellular profile observed in histological samples of the mechanically well-fixed acetabular components. We have previously shown that histiocytes and activated macrophages are abundant and lymphocytes absent in the tissue reaction around the cement/prosthesis complex [19].

In conclusion, we can report that, as analysed with human lymphocyte cultures, hydroxyapatite appears to be a relatively inert biomaterial and this is in accordance with previous clinical and experimental research. However, we doubt the theory that there could be chemical fixation or bony ingrowth into a hydroxyapatite-coated prosthesis component.

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