Osseointegration of polyethylene implants coated with titanium and biomimetically or electrochemically deposited hydroxyapatite in a rabbit model

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Abstract

Purpose The aim of this study was to evaluate the osseointegration of a new coating directly deposited on PE at room temperature.

Methods Thirty-six (36) male New Zealand rabbits were randomly assigned to receive one out of three types of implants: two tested implants, i.e. PE implant coated with TiPVD and biomimetic HA (biomimetic), PE implant coated with TiPVD and electrolytic HA (electrolytic), and positive control made of massive microrough titanium coated with plasma sprayed HA (TiHAPS). Osseointegration was evaluated by histomorphometry (bone tissue in contact [BIC]), mineralized bone area [MBA] and mechanical testing (push-out test, interfacial shear strength [ISS]) at six and 12 weeks in the distal femurs.

Results For BIC there were no differences between the groups at six ($p=0.98$) and 12 weeks ($p=0.13$). For MBA, no statistically significant difference was measured between groups at six ($p=0.52$) and 12 weeks ($p=0.57$). At six weeks, interfacial shear strength (ISS) was significantly higher ($p=0.01$) for TiHAPS implants compared to biomimetic and electrolytic implants. This difference was not significant at 12 weeks ($p=0.92$).

Conclusion The osseointegration of biomimetic and electrolytic implants was equivalent to a positive control at 12 weeks.

Keywords Osseointegration · Polyethylene implant · Biomimetic deposition · Electrolytic deposition · Hydroxyapatite coating · Rabbit model · Hip arthroplasty

Introduction

A stable secondary fixation obtained through osseointegration of cementless acetabular components is a key factor for the survival of cementless total hip arthroplasty (THA) [1]. Although polyethylene (PE) is the most common biomaterial used in THA, its fixation to bone tissue remains challenging and requires an additional interface between bone and the PE implant.

To improve the osseointegration of modular metal-backed acetabular components, hydroxyapatite (HA) has been introduced as a coating on the metal shell [2]. In vivo studies have shown that HA increased the interfacial strength between bone and implant. The most usual technique for HA coating is plasma spray deposition requiring high temperatures to spray the coating, that can alter the HA structure and lead to a poor adhesion of the HA coating on the substrate. In addition, plasma spray coatings have been incriminated to induce degradation and delamination of the HA coating, periprosthetic osteolysis and third-body wear [3].

As an alternative, biomimetic and electrolytic methods have been proposed for HA deposition [4, 5] with bone bonding properties comparable to that of plasma-sprayed HA [6]. However, direct HA deposition on PE is not possible with either method. Indeed, direct biomimetic HA deposition is technically possible but could lead to modification of the composition of the crystalline solution, with unknown effects on PE properties [7]. With the electrolytic method, the use of an osteoconductive material is necessary prior to HA deposition. Also, since HA coatings are resorbable materials, it is necessary to have an unresorbable and osteoconductive

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coating under HA to allow for a long-term bone fixation. In the present study, we have developed titanium (Ti) deposition performed at room temperature on polyethylene by ionic plasma, to preserve PE properties. This type of deposition allows for a final unresorbable and osteoconductive coating with a microroughness directly deposited on polyethylene implant. A second coating using hydroxyapatite (HA) deposited either biomimetically or electrochemically has been added to the titanium coating.

We hypothesized that this new dual coating would provide osseointegration comparable to bulk micro-rough titanium implants coated with plasma sprayed HA. Osseointegration was assessed by histomorphometric and biomechanical techniques in a rabbit model of osseointegration.

Materials and methods

Implants

Polyethylene and titanium implants used in this study were 6-mm long cylinders with 4.5-mm outside diameter. Titanium implants were coated by plasma-sprayed HA. Polyethylene implants were dual-coated with titanium and HA. The implants were sterilized using ethylene oxide (EtO), and were kindly provided by FH Orthopaedics (France, Quimper).

Polyethylene implants were first coated at room temperature by the ionic plasma vapour deposition of titanium (plasma vapour deposition, PVD) technique that consists of an atomistic deposition process in which the material being deposited is vaporized from a solid source (Ti6Al4V) in the form of atoms or molecules. Then, it is transported in the form of vapour through a vacuum or low-pressure gaseous environment to the substrate (polyethylene) where it condenses. Then these implants were coated with either biomimetic or electrolytic HA. All PE implants also contained a thin titanium axis to ensure rigidity.

Biomimetic HA

The hydroxyapatite coating is formed by precipitation of calcium phosphate on the surface of implants (polyethylene TiPVD coated). Calcium phosphate ions (CaP) are soluble in an acid environment. By increasing the solution pH, CaP precipitate on the surface implants. Stages of deposition are: preparation of implants by ultrasonic cleaning and immersion in a solution of implants nucleation at 37 °C. Immersing in a solution of crystal growth at 50 °C did the growth of the hydroxyapatite layer on the implants. HA coating thickness is about 30 µm.

Electrolytic HA

Implants are immersed in a crystalline solution with $\text{Ca}(\text{NO}_3)_2$ (0.2 g/2 l) and $\text{NH}_4\text{H}_2\text{PO}_4$ (0.08 g/2 l) for six hours at 90 °C. Electric current is applied (<10 mA, 1.3 V) by two other electrodes and a thin CaP coating (20 µm) is formed.

Animals

Thirty-six male adult New Zealand rabbits (Charles Rivers, France) were used. Animals were three months old, and checked for closure of the growth plate. Animals were housed individually in metal hutches with an ambient temperature of 21 °C and with 50 % air humidity according to the European guidelines for care and use of laboratory animals (Directive du Conseil 24.11.1986, 86/609/CEE). Artificial lighting was used to maintain a normal day/night rhythm. The animals were fed with water and commercial (Pietremnt, Sainte Colombe, France) food concentrates ad libitum. Study was approved by the Ethics Committee for animal experimentation of Lariboisière Hospital, University Paris VII, Paris, France (CEEALV/2011-11-02).

Surgical procedure

General anaesthesia was performed using intramuscular injection of ketamine hydrochloride (100 mg/kg), medeto-midine hydrochloride (0.25 mg/kg) and diazepam (0.5 mg/kg). Prophylactic antibacterial treatment consisted of Enfloxacine at a dose of 10 mg/kg. After induction of anaesthesia, the rabbit was surgically prepared and both lower limbs were draped for operation. A longitudinal skin incision was made over the medial femoral condyle to expose the distal aspect of the femur. A 6-mm long and 4.5-mm diameter defect was hand-drilled in the coronal plane. The cavity was evacuated with physiological saline, and packed with gauze until bleeding had subsided. Designated implants were tightly impacted (interference-fit). The wound was closed in layers. After six or 12 weeks the rabbits were sacrificed using an overdose of pentobarbital.

Experimental design

Each of the 36 rabbits had both lower limbs operated upon during the same intervention. A total of 72 defects were randomly assigned to one of the following groups: titanium coated by plasma-spray HA (TiHAPS), Ti-PVD and biomimetic HA coated polyethylene (biomimetics), Ti-PVD and electrolytic HA coated polyethylene. Six implants per group and per test at each time period were available for analysis (Table 1).

Table 1 Number of implants tested for each time and test

Number of implants evaluated at each time point	Biomimetic <i>N</i> =24	Electrolytic <i>N</i> =24	TiHAPs <i>N</i> =24
Six-week histology	6	6	6
Six-week compression	6	6	6
12-week histology	6	6	6
12-week compression	6	6	6

Histomorphometric evaluation

Each bone tissue specimen ($n=36$) was fixed in 10 % phosphate-buffered formalin, rinsed in water, dehydrated in ethanol, cleared in xylene and embedded in methyl-methacrylate. The femoral condyles were sectioned perpendicular to the long axis of the implant using a circular water-cooled diamond saw (Microcut, Brot, France). Each section was then grounded down to a thickness of about 150 μm . The surface of these preparations was stained with Stevenels' blue and van Gieson picro-fuchsin for subsequent standard light microscopy and histomorphometric analysis. Three sections per condyle were analysed by histomorphometry. For each section, two parameters, the percent of bone tissue in contact with the implant (BIC) and the percent of mineralized bone area (MBA) in the circumferential zone (100 μm) around the implant were determined. Measurements were made using NIS Elements software (Netherlands, Amsterdam) in conjunction with an image processing system consisting of a microscope and a video camera (Nikon, Eclipse TE2000-U; Nikon Digital camera DMX1200F). BIC was calculated from the sum of the areas where bone was in contact with each implant. Briefly, the image (magnification $\times 2$) was digitized, a circle was drawn at the implant perimeter, then the arcs where the bone was in direct contact were selected and the corresponding angles were measured (in degrees) from the centre of the circle. Bone implant contact (BIC) was defined as the sum of the angles, expressed as a percentage out of 360°. In order to calculate the MBA, each histology image (magnification $\times 2$) was digitized and a ring (100- μm wide) was delineated around the perimeter of each implant. The fraction of this annular area, which was covered by mineralized tissue, was measured and expressed as the percent of the total tissue area.

Mechanical testing

Thirty-six bone tissue specimens excised from the distal femurs were used for mechanical testing. The distal femurs were harvested 'en bloc' and were stored at $-20\text{ }^{\circ}\text{C}$ until the push-out testing. They were thawed 24 hours before the test at $4\text{ }^{\circ}\text{C}$. All mechanical testings were performed using an Instron

(4,505 bend top test machine fitted with a one kilo Newton (kN) load cell). Fixation was assessed by calculating the interfacial shear strength (ISS) using push-out testing as follows:

$$\text{ISS(MPa)} = \text{Maximal load (N)} / \Pi \times D(\text{mm}) \times L(\text{mm}).$$

Tests were performed on sections of retrieved bone cut to expose the flats ends of the implants, using a specially designed jig to ensure the correct application of force.

Statistical analysis

Statistical analysis was determined by a non-parametric test (Mann–Whitney) using Graph Pad Prism 5 (5.01 version). Significance was defined as a *p* value of less than 0.05. The differences between groups at each time point of histomorphometric and mechanical parameters were assessed over the 12-week period using an ANOVA analysis (StatGraphics Centurion XV, version 15.2).

Results

Histological findings

No fibrosis tissue was found around any implants. Bone formation around implants was seen with increasing maturation process between six and 12 weeks. No resorption of HA coating was seen in any group (Fig. 1).

Histomorphometric findings

At six weeks, the BIC mean values were 0.61 ± 0.19 , 0.65 ± 0.13 and 0.65 ± 0.12 for TiHAPS, biomimetic and electrolytic implants, respectively. At 12 weeks, the BIC mean value was 0.63 ± 0.18 for TiHAPS, 0.74 ± 0.08 for biomimetic and 0.61 ± 0.12 for electrolytic. There was no significant difference between groups at six ($p=0.98$) and 12 weeks ($p=0.13$). There were also no differences between six and 12 weeks between the groups ($p=0.55$).

At six weeks, the MBA mean value ($\pm\text{SD}$) was 0.58 ± 0.17 , 0.59 ± 0.07 and 0.65 ± 0.11 for TiHAPS, biomimetic and electrolytic implants, respectively. At 12 weeks, the MBA mean value ($\pm\text{SD}$) was 0.53 ± 0.18 , 0.67 ± 0.09 and 0.61 ± 0.15 for TiHAPS, biomimetic and electrolytic implants, respectively.

No statistically significant difference was measured between groups at six ($p=0.52$) and 12 weeks ($p=0.57$), respectively. There was no difference between six and 12 weeks between the groups ($p=0.45$).

Histomorphometric results are summarized in Table 2.

Fig. 1 Histological findings for electrolytic implant at six (*top*) and 12 weeks (*bottom*). Bone maturation between six and 12 weeks without fibrosis interposition between bone and implant (I). Same results were found with TiHAPs and biomimetic implants

Interfacial shear strength (ISS)

The interfacial shear strength (ISS) at six weeks was 7.41 ± 1.64 , 4.01 ± 1.53 and 4.14 ± 1.57 MPa for the TiHAPs, biomimetic and electrolytic implants, respectively. There was a significant difference between groups at six weeks ($p=0.01$). Fracture always occurred at the bone-to-coating interface for all implants. At 12 weeks, the interfacial shear strength (ISS) measured 5.07 ± 1.87 , 5.33 ± 2.61 and 4.66 ± 1.63 MPa for TiHAPs, biomimetic and electrolytic implants, respectively. There was no difference between groups at 12 weeks ($p=0.92$). The fracture area was entirely located between bone and coating for TiHAPs implants, and partially for biomimetic and

electrolytic implants, which also presented a fracture area between coating and PE at the distal part of the implants. There were no differences in the ISS results between six and 12 weeks for the three groups ($p=0.06$). Mechanical findings are summarized in Fig. 2.

Discussion

Implant osseointegration is defined by an intimate bone contact and high adhesive interfacial shear strength. In the current study, histomorphometric evaluation was selected because it is a reliable and well-defined method for which considerable work has been performed and numerous historical data are available [6, 8–10].

This study demonstrated that polyethylene implants coated with Ti-PVD and biomimetically or electrolytically deposited HA were able to develop osseointegration comparable to that of a plasma sprayed-HA coated titanium PE implant, that are largely used in hip arthroplasty.

Histomorphometric data did not show any differences between implants for bone tissue in contact and mineralized bone area between the six- and 12-week time points. These data could be interpreted as the indication that mineralization was almost complete at six weeks in this model. The histological difference between six and 12 weeks was mostly related to bone remodeling, more evident at 12 weeks. Bone

Table 2 Bone tissue in contact (BIC) and mineralize bone area (MBA)

Implants	BIC	MBA
Six weeks		
TiHAPs	0.61 ± 0.19	0.58 ± 0.17
Biomimetic	0.65 ± 0.13	0.59 ± 0.07
Electrolytic	0.65 ± 0.12	0.65 ± 0.11
12 weeks		
TiHAPs	0.63 ± 0.18	0.53 ± 0.18
Biomimetic	0.74 ± 0.08	0.67 ± 0.09
Electrolytic	0.61 ± 0.12	0.61 ± 0.15

Results are expressed as the ratios over the total contact area (mean $\pm$ standard deviation)

Fig. 2 Interfacial shear strength (ISS) in MPa at six and 12 weeks

remodeling has been described to increase up to 52 weeks in rabbits [11].

The only measurable difference between TiHAPS, biomimetic and electrolytic implants was a greater mean ISS value for TiHAPS implants on the push-out testing at six weeks. This difference was no longer apparent at 12 weeks.

The coating combination of a thin titanium layer (Ti-PVD) and biomimetically or electrolytically deposited HA was therefore comparable to bulk metal titanium implants coated with plasma sprayed HA with regards to mechanical properties and the observable development of osseointegration at 12 weeks.

The present study has some limitations. First, the method used to produce the HA coating could influence the long-term stability of the implant. In a rabbit model in which implants were inserted in the cortical bone, the comparison of plasma sprayed HA-coated and electrolytic HA-coated titanium implants showed greater values of ISS for plasma sprayed HA-coated implants at six and 12 weeks but not at 52 weeks where ISS was even significantly lower than for electrolytic HA-coated implants. The authors explained these results by a slower bone bonding associated with a lower rate of fibrosis with electrolytic HA coating [6, 11]. Furthermore, it has been also assumed that biomimetic HA deposition could limit the coating delamination. This property would be related to the well controlled chemical and physical conditions (pH, temperature and CaP composition of SBF) during HA deposition, which leads to a thinner and more resorbable HA coating than plasma sprayed deposition [10, 12]. Finally, even if no difference emerged between electrolytic and biomimetic HA-coated implants in our study, electrolytic HA deposition has been reported to be more favourable than biomimetic deposition to osseointegration, based on a higher interfacial shear strength

12 weeks after implantation, while no differences in the mineralized bone area was found between the two methods of HA deposition at six and 12 weeks [3]. The only limitation to expand the conclusions of these studies to the present one is that these studies evaluated massive titanium implants which have been shown in vitro to promote osteoblast adhesion, a phenomenon not described with plasma sprayed HA coated PE implants.

The variations of the interfacial shear strength between six and 12 weeks in the titanium HA coated implants (TiHAPS) remain unclear. It was verified that this was not due to outlying values in some animals. Another possibility would be the relative differences in the surface characteristics of TiHAPS and the other implants. In vitro studies showed that the surface characteristics like roughness, porosity, coating particles size and especially stiffness of implants influence the osteoblast's function and subsequently the formation of new bone [13, 14]. It could be envisioned that TiHAPS may have triggered greater bone formation within the first six weeks, which afterwards has stabilized or has been caught up by the bone formation around the electrolytic and biomimetic implants.

Furthermore, despite the precautions taken to reproduce the same condition of the mechanical tests, with the use of the same cut system and push out testing for all implants, the Young's modulus of the tested implants (biomimetic and electrolytic deposited HA- and thin titanium layer-coated PE implants) were different from those measured in the control TiHAPS implants. Therefore, the stress distributions were applied differently in the three groups of implants [15].

Finally, the microrough, plasma sprayed HA-coated titanium implant selected as control is debatable, because it was made of non-porous titanium. The reason behind this selection was that these titanium implants had the same roughness as the

tested implants after coating deposition. They were considered a better control than porous titanium implants despite that porous titanium demonstrated greater osseointegration than rough titanium implants because of a larger area in contact with bone (bone ingrowth) [16].

The present study demonstrated that the combination of a thin and non resorbable TiPVD with a biomimetic or electrolytic HA coating was favourable to the bone integration of PE implants. These implants could theoretically prevent the occurrence of complications related to modularity, including backside wear.

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