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Hydroxyapatite-coated orthopaedic screws as infection resistant materials: in vitro study

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Abstract

The authors evaluated in vitro the adherence of a *Staphylococcus epidermidis* strain to hydroxyapatite-coated stainless-steel screws—used in orthopaedic surgery for external fracture fixation—in comparison with the adherence to uncoated screws. Evaluations were also performed on analogous groups of screws immersed for 72 and 168 h in a solution at 37°C, in order to simulate the interstitial fluid in a simplified way. Adherence values on coated prostheses resulted significantly lower compared with those observed on metal prostheses, both in basal conditions and after immersion in saline solution. Moreover, both on coated and on uncoated screws a significant reduction in bacterial adherence was noted related to the duration of the prosthesis permanence in saline solution.

Keywords: Hydroxyapatite; Stainless steel; Orthopaedic screws; Bacterial adherence; Infection resistant material

1. Introduction

The main obstacle to a wider use of biomedical implants is the high probability of prosthesis-associated infection. The leading role of biomaterial–bacteria interactions has been emphasized [1–4]. The material's surface and the biological environment's features affect, as a matter of fact, the adhesive behaviour of micro-organisms.

In order to reduce the incidence of prosthesis-associated infections, several biomaterial surface treatments have been proposed. It has been, moreover, inferred that infections should be controlled not only by direct inhibition of bacterial adherence, but also by enhancement of tissue compatibility or integration.

A metal prosthesis' surface treatment with hydroxyapatite (HA) was recently devised in order to promote integration between bone tissue and prosthesis. Clinical studies performed on implants, up to now, highlight how HA-coating improves the bone–biomaterial interface, conferring therefore on the prostheses good osteointegration capabilities. Some studies suggest that use of HA-coated prostheses is related to a lower infection incidence [5]. In prostheses, more exposed to the risk of contamination, such as screws for external fixation, this behaviour could result extremely advantageous.

In the present study, the adherence of a Staphylococcus strain to HA-coated stainless steel screws has been evaluated in vitro as compared with the adherence measured on uncoated stainless steel screws. Hydroxyapatite is a highly bioactive material which rather quickly undergoes surface modifications after implantation. Adherence was also evaluated after prolonged immersion of the screws in saline, in order to modify the surface properties as it happens in vivo. Actually, Kummer et al. [6] demonstrated increased instability of HA-coatings when tested in physiological solutions; Radin et al. [7] studied the dissolution of HA in simulated interstitial fluid. Surface reactivity, i.e. capability to interact in vivo with

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surrounding tissues and fluids as related to bone formation and bone tissue bonding, was evaluated by Ducheyne et al. [8] by means of zeta potential measurement in several physiological solutions; these measurements act as a suitable method to assess the actual state of the solid solution interface in situ. In our study HA-coating modifications were evaluated by determining Ca and P concentrations in the medium in relation to immersion time. Similarly, the release of iron, chromium and nickel ions from the uncoated screws was evaluated.

2. Materials and methods

Sixty stainless steel external fixation tapered screws were used: thirty screws were plasma sprayed with HA while the others remained uncoated (Orthofix, Bussolengo, Italy). Aside from the surface modification, the HA-coated screws were identical, as for size and style, to the all-stainless steel screws. Both coated and uncoated prostheses were divided into three groups: the first one underwent no treatment, the second one was kept in saline solution at 37°C for 72 h, whereas the third one was kept in saline solution at 37°C for 168 h.

In the incubation medium of HA-coated screws, calcium concentration was evaluated by means of a flame atomic absorption spectrophotometer PU 9400 (Philips).

Phosphorus concentration was evaluated by means of the automated UV-test for BM/Hitachi System 717 (Boehringer-Manneheim).

In the incubation medium of uncoated screws the concentration of iron, chromium and nickel ions was evaluated by means of a flameless atomic absorption spectrometer (ATI-Unicam QZ 939). AAS standard solution (Sigma) containing 1000 mg Fe/L, ultrapure water (Baker Instra) and sterile saline solution was employed for iron determination. In the incubation media of both screw types the pH was evaluated in a calibration curve with the super aution analyzer SA-4220 (DIC-Kyoto).

A strain of *Staphylococcus epidermidis*, collected from a removed hip prosthesis, was used for adherence tests.

The screws were exposed in vitro to predetermined bacterial suspensions (10^6 CFU ml^{-1}) in trypticase soy broth, then washed until the washing liquid was sterile, and finally incubated in a sterile culture medium at 37°C for 24 h. After having removed the screws from the broth, the broth rich in bacteria was examined. Quantitative analysis of Staphylococci adhered to the screws was carried out by evaluating the broth bacterial concentration (in nefelometric turbidity units, NTU) by means of a turbidimeter (Ratio/XR Turbidimeter, Hach) [2, 9, 17–19].

The adherence tests were performed:

(a) on coated and uncoated screws exposed to air;

(b) on coated and uncoated screws kept for 72 h in NaCl 0.9%, then removed from the solution, washed with saline and reincubated in fresh medium;

(c) on coated and uncoated screws kept for 168 h in NaCl 0.9%, then removed from the solution, washed with saline and reincubated in fresh medium.

3. Results

The results of the quantitative evaluations on adherence obtained in the single experiments performed on different screw types are shown in Table 1.

The mean adherence value, expressed in NTU, obtained with uncoated screws is 118.61 ± 4.47. The

Table 1
Adhesion values in the single experiment performed on different groups of screws

Uncoated			HA-Coated		
Untreated	72 h-treated	168 h-treated	Untreated	72 h-treated	168 h-treated
115.5	115.5	109.5	117	100.5	96.75
126	105	105.75	116.25	100.5	96.75
112.5	107.25	105.75	106.5	100.5	96.75
116.25	108	106.5	110.25	97.5	96
118.5	109.5	104.25	108	97.5	96.75
124.5	108	109.5	105	98.25	99
118.5	117.75	108	109.5	102	96.75
123	111.75	108	108	102	99
114.75	108	108	109.125	102	99.75
116.65	//////	108	110.45	100.5	99.75
(M.V. ± SD) 118.61 ± 4.47	(M.V. ± SD) 110.75 ± 4.87	(M.V. ± SD) 107.325 ± 1.71	(M.V. ± SD) 110 ± 3.86	(M.V. ± SD) 100.12 ± 1.77	(M.V. ± SD) 97.72 ± 1.46

Table 2
Significance of the differences among the adhesion values of the HA-coated screws (student's *t*-test)

Screw type	Exp. no.	Mean ± SD	*P*
HA-coated untreated	10	110 ± 3.86	
HA-coated 72 h treated	10	100.12 ± 1.77	0.0001
HA-coated 168 h treated	10	97.72 ± 1.46	0.004

Table 3
Significance of the difference among the adhesion values of the uncoated screws (student's *t*-test)

Screw type	Exp. no.	Mean ± SD	*P*
Uncoated untreated	10	118.61 ± 4.47	
Uncoated 72 h treated	9	110.75 ± 4.87	0.0019
Uncoated 168 h treated	10	107.32 ± 1.71	0.0521

mean adherence value obtained with uncoated screws exposed to saline solution at 37°C for 72 h is 110.75 ± 4.87, the one obtained with HA-coated screws exposed to saline solution at 37°C for 168 h is 107.325 ± 1.71.

The mean adherence value, expressed in NTU, obtained with HA-coated metal screws not exposed to saline solution is 110 ± 3.86. The mean adherence value obtained with HA-coated screws exposed to saline solution at 37°C for 72 h is 100.12 ± 1.77, the one obtained with HA-coated metal screws exposed to saline solution at 37°C for 168 h 97.72 ± 1.46.

Tables 2 and 3 shows the results of the statistical comparison (Student's *t*-test) performed with three groups of HA-coated screws and with three groups of uncoated screws.

The comparison between HA-coated screws before and after immersion in saline solution demonstrates that the immersion in saline for 72 h induces a statistically significant reduction in adherence ($P = 0.0001$), and that the immersion in saline up to 168 h induced a further reduction still statistically significant ($P = 0.004$), even if at a lower level of significance.

The comparison between uncoated screws before and after the immersion in saline solution for 72 h demonstrates a statistically significant reduction of adherence ($P = 0.0019$). Immersion in saline up to 168 h gives a further reduction in adherence, not however statistically significant ($P = 0.052$).

Table 4
Evaluation in the extraction fluid of the calcium and phosphorus concentration from HA-coated screws

	Calcium	Phosphorus	pH
Saline (0.9% NaCl)	0	0	5
After 72 h in saline	10.94	0.93	7
After 168 h in saline	11.92	0.81	7.2

Table 5
Evaluation in the extraction fluid of iron, chromium and nickel concentration from uncoated screws

	Iron (ppm)	Nickel (ppm)	Chromium (ppm)
Saline (0.9% NaCl)	0	0	0
After 72 h in saline	13.05	24.23	7.56
After 168 h in saline	15.9	31.08	7.17

Table 6
Significance of the differences among the adhesion values of HA-coated and uncoated screws (student's *t*-test)

Screw type	Exp. no.	Mean ± SD	*P*
Uncoated untreated	10	118.61 ± 4.47	0.0002
HA-coated untreated	10	100.12 ± 1.77	
Uncoated 72 h treated	9	110.75 ± 4.87	0.0001
HA-coated 72 h treated	10	100.12 ± 1.77	
Uncoated 168 h treated	10	107.32 ± 1.71	0.0001
HA-coated 168 h treated	10	97.72 ± 1.46	

Table 4 shows that the maximal calcium release is reached at 72 h, when a highly significant reduction of bacterial adhesion is observed.

With uncoated screws the maximal ion release is also observed at 72 h, when a significant reduction in bacterial adhesion is observed. After 168 h of immersion in saline no further ion release occurs and the bacterial adhesion does not significantly decrease (Table 5).

Table 6 shows the results of the statistical comparison (Student's *t*-test) between HA-coated and uncoated screws both in basal conditions and after immersion in physiological solution. Bacterial adherence on HA-coated screws is significantly lower when compared to untreated screws, in basal conditions ($P = 0.0002$), after 72 h ($P = 0.0001$) and after 168 h ($P = 0.0001$).

4. Discussion and conclusions

Orthopaedic prostheses may play a role in the pathogenesis of infections following surgery, and the implant surface properties may influence the adherence of Staphylococci to prosthetic materials [1–3, 9]. Finishing the material surface with a repellent coat to reduce bacterial adherence can reduce the risk of developing an infection.

Many studies were focused on the search for coatings able to make the material repellent to bacterial adherence. Surfaces of biomaterials soaked with antibiotics, or coated with polyacrylamide films bound to antiseptic substances, or with quaternary amines containing organosilicon salts have shown antibacterial properties [10–13].

Staphylococcus adherence on polystyrene was limited by modifying artificial surface with surfactants [14]. A significant reduction in bacterial adherence was noted on polyethylene terephthalate surface modified with polyethylene oxide [15]. Also biologic molecules, such as heparin, were successfully used for this purpose [16–19]. Most of the above listed treatments were performed on polymers, but also metal surfaces can be modified. Dunkirk et al. [16], for example, have proposed photochemical coupling process both on polymers and metals for the prevention of bacterial colonization.

Opalchenova et al. have demonstrated in vivo [20] and in vitro [21] a significant antimicrobial effect of calcium phosphate ceramics.

In our study we carried out a quantitative evaluation of the adherence of a strain of *Staphylococcus epidermidis* on two types of screws used for external fixation in orthopaedic surgery, one in hydroxyapatite-coated stainless steel and the other in uncoated stainless steel. Bacterial adherence on HA-coated screws resulted significantly lower than on uncoated screws. Moreover, adherence on both screw types (HA-coated and uncoated) decreased significantly after immersion in saline solution.

Adherence decreases significantly after immersion in saline for 72 h and further decreases, though in a less evident way, after 168 h, both in HA-coated and uncoated screws. The adherence difference between HA-coated and uncoated screws remains however constant (HA-coated = uncoated).

These observations point out that both surface properties and their changes after immersion in saline solution influence bacterial adherence.

Surface properties can therefore favour in vivo the material-tissue integration, and at the same time can limit materials infectibility.

In the specific case of the HA-coated screws examined in this study, if it is assumed that the Ca and P release is representative of the material tendency to get integrated with bone tissue, and that such tendency matches with the tendency to hamper bacterial adherence, it can be inferred that the most refractory surface to bacterial adherence is the one which allows the maximum prosthesis integration.

It can be therefore hypothesized that HA-coating limits infectibility both by favouring the material–tissue integration and reducing bacterial adherence.

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