Adult human bone cells from jaw bones cultured on plasma-sprayed or polished surfaces of titanium or hydroxylapatite discs

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Adult human bone cells isolated from jaw bone biopsies were cultured either on Thermanox® coverslips or on polished or plasma-sprayed surfaces of titanium or hydroxylapatite and the levels of their various metabolic functions were compared after 2 and 5 days of staying in culture. Thus, jaw bone cells grown on hydroxylapatite proliferated very little, while expressing discrete levels of alkaline phosphatase activity and of osteocalcin secretion into the growth medium. On the other hand, bone cells seeded onto titanium surfaces proliferated much more intensely than those on Thermanox®, besides expressing alkaline phosphatase (very intensely after 5 days) and secreting osteocalcin. Thus, both kinds of titanium surfaces greatly enlarged the size of both populations of pre-osteoblastic precursors and of pre-osteoblasts *in vitro*, but plasma-sprayed titanium surfaces elicited, between day 2 and 5 in culture, greater increases in bone cell numbers markedly enhancing their proliferative and alkaline phosphatase activities, along with their osteocalcin secretion into the growth medium, and thus favouring the expression of the mature osteoblastic phenotype. These preliminary findings show that studies correlating the physical surface features of various biomaterials with the corresponding expression of specific differentiation markers by the bone cells cultured on these same surfaces can provide information relevant to the clinical application of biomaterials.

1. Introduction

Surface roughness (SR) is believed to be an important factor among those known to be capable of inducing an optimal bone neoformation around implants [1]. The results of several investigations into the relationship between physical surface features and *in vitro* or *in vivo* cyto-compatibility of various biomaterials currently employed in dental or orthopaedic practice have led to the idea that uneven surfaces might elicit a better local cellular response than smooth surfaces do. In this respect, the chemical nature of biomaterials may have a fundamental role in affecting the character of the biological response. Among the wide range of currently available biomaterials, those most frequently used in clinical settings are hydroxypatite (HA) and commercially pure titanium (Ti) [2–4]. The purpose of the present research was to carry out a detailed analysis of the *in vitro* behaviour of adult human osteoblasts grown out of jaw bone biopsies and cultured for up to 5 days on samples of either Ti or HA or on surfaces made up of plastic (i.e. Thermanox®). We tested samples of both HA and Ti that were endowed with either a very smooth (polished) or a craggy (plasma-sprayed) surface. The different morphological features of such surfaces could be characterized in detail and accurately quantified by both contact and microscopic methods. Thus, the data obtained on several specific functions of the cultured bone cells could be correlated with the physical features of identical unit surface areas of biomaterials on which the adult human jaw bone cells were grown.

The results herein reported show that titanium surfaces, particularly the plasma-sprayed ones, are endowed with advantageous features that make them more suitable to successful clinical applications in the field of bone implants.

2. Materials and methods

2.1. Biomaterials

Calcium phosphate ceramic (hydroxylapatite, HA) and titanium (Ti) were supplied by Bio-Interfaces Inc. (San Diego, CA). Both HA and Ti samples tested were shaped as discs 10 mm in diameter and 1 mm thick. The

surface of part of the HA or Ti discs was pre-treated with a plasma-spray coating (PS) of either HA or Ti microparticles, respectively. The surface of the remaining HA or Ti discs underwent a thorough smoothing treatment with polishing powders. Once formed, the discs were packed, sealed and sterilized by gamma-irradiation at their sites of production. The *in vitro* studies with adult human bone cells isolated from jaw bones were thus carried out on: (i) smooth-surfaced or polished HA (HA-pol); (ii) plasma-sprayed HA (HA-PS); (iii) polished Ti (Ti-pol); and (iv) plasma-sprayed Ti (Ti-PS). Moreover, as a reference control, human jaw bone cells were cultured in parallel on a plastic polymer (Thermanox®; Nunc Inc.).

2.2. Physical surface features of biomaterials

The surface configuration of the four types of metal discs used was characterized using the following procedures:

(i) *Profilometry* by a bi-dimensional contact method (2d roughness; 2*dR*). The 2*dR* was estimated by means of a Taylor–Hobson profilometer equipped with a 4 mm diameter tip pyramidal stylus. At least nine measurements were carried out for each type of biomaterial tested as the standard deviation of the data did not significantly change after 9–10 measurements [5]. During each test, the examiners wore cotton gloves in order to prevent both scratches and any direct skin contact with the surfaces of the discs. According to the size of the disc, a sampling length of 4.0 mm was selected. The roughness parameters chosen for quantifying the 2*dR* were: R_a (the arithmetic mean of the roughness height distances); R_t (the distance between the top of the highest peak and the bottom of the deepest valley); R_{tm} (the average of five consecutive values of roughness height); and S_m (the arithmetic mean of groove distances) [6]. All these parameters were expressed in micrometres (μm) [1].

(ii) *Scanning electron microscopy* (SEM) gives a direct close-at-hand image of the peaks and valleys bi-dimensionally characterized by the profilometer. SEM analyses were performed using a Cambridge 604 Microscope in both Se and Bse mode, 1–2 kV, at ×100, ×1000 and ×3000 magnifications. Plasma-sprayed discs were also split prior to testing in order to verify the uniformity and thickness of the coatings.

(iii) *Confocal laser scanning microscopy* (CLSM) is a tri-dimensional method (3d roughness; 3*dR*) [5, 6]. The confocal laser scanning microscope is an optical, i.e. non-contact profilometer that can characterize surfaces by means of a laser beam. For imaging purposes, a LaserTec microscope (LaserTec Corporation, London, UK) was used. The sample discs were moved in a pre-defined direction while their surface was illuminated by the laser source. After encountering the disc surface, the laser beam was reflected in the *x*, *y*, and *z* planes as a function of the actual degree of surface roughness. The reflected light was detected by sensors and used as a data matrix of the surface tested. The *SR* thus reconstructed was shown in a 3D-profile display with the different valley depths displayed in a 256 grey-scale or colour-scale mode. For each sample, the scanned area was 111.54 μm long and 89.78 μm wide, corresponding to an apparent surface area (ASA) of 10014.1 μm^2. This methodology is an outstanding tool as it makes it possible to calculate the real surface area (RSA) per unit of ASA as a function of the asperity of the biomaterial examined [5, 6].

(iv) The chemical composition and the degree of crystallinity of the HA discs were assayed by X-ray diffraction crystallography (XRDC) [7]. An X-ray generator (Diffractometer Rigaku-Miniflex, CuK_α-radiation, C.N.R. IRTEC, Faenza, Italy) was used to obtain XRDC patterns for the HA discs. The percentage degree of crystallinity and the *a*, *b* and *c* lattice parameters were calculated from these patterns.

2.3. Adult human jaw bone cell cultures

Tiny fragments of jaw bones, biopsied during surgical procedures carried out on several 16 to 22 year-old patients, were aseptically rinsed twice or thrice in PBS/A solution (Oxoid). After undergoing two predigestion steps of 45 min each at 37 °C with collagenase type II solution (0.5 mg/ml; Sigma Chemical Co.) [8], the bone shreds were minced by means of bone cutters and plated into 12-well "cluster" tissue culture dishes (Costar). Each well in these dishes contained DMEM medium (ICN-Flow) fortified with heat-inactivated fetal bovine serum (10% v/v; FBS (ICN-Flow)), sodium ascorbate (50 μg/ml; Sigma), and a supplement of antibiotics (penicillin G sodium, amphotericin B, and streptomycin sulfate, all from Gibco) [9]. After 2 weeks in culture, the outgrowths of bone cells surrounding each bone fragment were detached by trypsinization (trypsin 0.25% w/v; Gibco), and subsequently seeded onto standard plastic tissue culture flasks (F75; Falcon). Once the secondary cultures had become sub-confluent, which happened within one week, the adult human osteoblasts were again detached by trypsinization, and next used for experimental purposes. The isolated bone cells were seeded onto biomaterial discs of four kinds (HA-PS, HA-pol, Ti-PS, and Ti-pol) and on Thermanox® discs at a density of 50 000 cells/disc. Each of these discs was placed inside one well of a 24-well "cluster" tissue culture dish (Costar), and 100 μl of the cell suspension was applied with great care onto the upper surface of each disc in order to avoid any unwanted spilling and, thereby, attachment of the bone cells to the surrounding plastic surface of the well. The bone cells were allowed to attach to the upper surfaces of the various discs for 3 hours at 37 °C in air with 5% v/v CO_2 added; thereafter, 1 ml of growth medium was also cautiously added to each well [10]. This medium contained β-glycerophosphate (10 mM), and its addition coincided with the actual onset of the experimental treatments. Thereafter, the growth medium, always supplemented with the same lot of FBS, was changed every 2 days. Wells containing bone cells growing on plastic in addition to the biomaterial discs—an infrequent occurrence—were not used for experimental purposes. The functionally specific parameters exhibited by the human bone cells seeded onto

discs made up of either HA or Ti or plastic were evaluated in parallel both 2 and 5 days after the start of the experiments.

2.4. Functional parameters of cultured adult human jaw bone cells

(i) *Alkaline phosphatase* (ALP). The ALP activity of the *in vitro* bone cells was evaluated by both biochemical and cytochemical methods. Quantitative determinations on 100 µl samples of cell lysates were carried out according to the procedure suggested for the Sigma kit no. 104 (Sigma). On day 2 and 5, *in vitro* adult human bone cells that had been grown onto different biomaterials were rinsed twice in PBS/A and next lysed by an exposure for 30 min at 4 °C to 0.1% v/v Triton X-100. The lysed samples were manually homogenized in a Dounce homogenizer and then spun down: the supernatants thus obtained were assayed for ALP activity at 37 °C, using *p*-nitrophenyl phosphate as a substrate. The ALP activity values of the samples were calculated from their corresponding optical adsorbances at 410 nm and expressed as units of enzyme activity per mg bone cell protein (U/mg protein). Duplicate determinations were performed for each biomaterial tested and for the Thermanox® specimens. The ALP activity at the cell level was revealed by means of a cytochemical method in fixed (methanol at − 20 °C for 20 min) cell monolayers using naphtol AS-MX phosphate (Sigma) as a substrate in 2 mM Tris pH 8.6 [11].

(ii) *Osteocalcin*. Bone cell cultures on biomaterial and Thermanox® discs were rinsed twice in PBS/A and next incubated for 48 h, prior to termination, in serumless growth medium to which bovine serum albumin (BSA; 1% w/v) (Sigma) had been added instead of the FBS. The levels of osteocalcin in the 48 h cell-conditioned growth media were assayed by means of a specific radioimmunoassay (Cis Bio International) and expressed for each sample as ng osteocalcin/mg cell protein of corresponding cultures. The osteocalcin assays were carried out in duplicate for each biomaterial and the Thermanox® discs at each time point.

(iii) *DNA synthesis*. The new DNA synthetic activity in cultures of bone cells was evaluated by their macromolecular incorporation of [^{3}H]-thymidine ([^{3}H] TdR; 0.5 µCi/ml) during the last 24 h prior to sampling [12]. The cellular samples were exposed to tri-chloro-acetic acid (40% w/v) in 1.2 N HCl; the precipitates thus obtained were solubilized in 0.3 N NaOH, and their radioactivity was determined in a liquid scintillation counter (Canberra-Packard). The radioactivity values were expressed as cpm of macromolecularly incorporated [^{3}H] TdR/µg bone cell protein.

(iv) *Bone cell protein*. The protein content of the bone cell cultures was assayed according to the procedure of Lowry [13].

(v) *Assembly of type I collagen fibrils in vitro*. Cultures of adult human bone cells on plastic "cluster" wells (Costar) were rinsed with PBS/A, fixed with absolute methanol at − 20 °C for 20 min and, after masking any non-specific antibody-binding sites with bovine serum albumin (BSA; 2% w/v; Sigma), incubated overnight, at 4 °C with a rabbit polyclonal antibody (diluted 1: 100 in 2% w/v BSA solution) directed against type I collagen (Biodivision). Specifically bound antibodies were demonstrated by means of the peroxidase-anti-peroxidase complex (PAP) procedure in the presence of H_2O_2 and diaminobenzidine (Sigma).

(vi) *Calcification of extracellular matrix*. Adult human jaw bone cells previously grown on plastic "cluster" wells (Costar) in the presence of β-glycerophosphate were stained according to the method of von Kossa [14] to reveal the deposition of insoluble calcium phosphate salts in the extracellular matrix. To this purpose, bone cell cultures were washed with Tris-buffered saline (TBS), next fixed in methanol (90% v/v), and finally rinsed with water. Thereafter, an $AgNO_3$ solution (5% w/v) was poured into each well, and the cultures were exposed for 5 min to ultraviolet light prior to being fixed with $Na_2S_2O_3$.

2.5. Statistical analysis

Mean values, standard deviations (SD) and standard errors of the means (SEM) were computed for six patients at each of the time-points investigated. Student's *t*-test and the analysis of variance (ANOVA) were utilized to assess the level of significance of the differences between the mean values of the various experimental groups.

3. Results

3.1. Physical surface features of biomaterials

Profilometry investigations showed that the values of the R_a, R_t, and R_{tm} parameters were significantly different ($p < 0.0001$) for the HA or Ti plasma-sprayed surfaces and the corresponding polished surfaces (Table I). Conversely, the values of the R_a, R_t, R_{tm}, and S_m parameters did not significantly differ ($p > 0.05$) between the Ti-pol and HA-pol samples (Table I). Finally, the values of the R_a, R_t, and R_{tm} parameters were found to significantly ($p < 0.0001$) differ between Ti-PS and HA-PS samples, whereas the difference between the corresponding S_m values was not significant ($p > 0.05$) (Table I).

SEM imaging proved itself to be an ideal tool for visualizing the shapes of peaks and valleys in plasma-sprayed HA and Ti samples as contrasted with the flatness of the corresponding polished samples, in agreement with their bi-dimensional description obtained by profilometry (Figs 1 and 2).

The examination of the biomaterials by CLSM showed that the RSA values of plasma-sprayed Ti and HA samples were significantly different ($p < 0.02$) from those of the corresponding polished samples (Table II). By contrast, the differences between Ti-pol and HA-pol RSA values were found to be not significant ($p > 0.05$), whereas HA-PS and Ti-PS RSA values significantly differed ($p < 0.05$) (Figs 3 and 4).

TABLE I SR characterization of the several biomaterials investigated by profilometry parameters

Biomaterials	$R_a \pm SD$	$R_t \pm SD$	$R_{tm} \pm SD$	$S_m \pm SD$
Ti-pol	0.28 ± 0.06	2.67 ± 0.50	1.42 ± 0.33	284.54 ± 200.24
HA-pol	0.22 ± 0.26	1.97 ± 0.10	1.21 ± 0.66	105.71 ± 66.78
Ti-PS	5.43 ± 0.83	45.43 ± 10.29	32.44 ± 0.39	81.27 ± 9.91
HA-PS	3.60 ± 0.16	28.12 ± 2.92	21.93 ± 1.09	75.49 ± 11.58

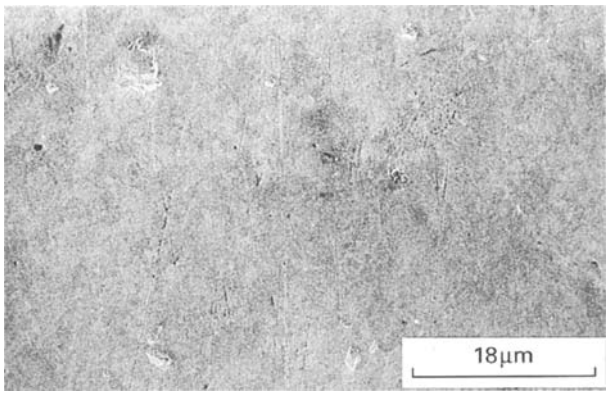

Figure 1 Scanning electron micrograph of HA-pol surface confirming the smoothness of HA-pol surfaces.

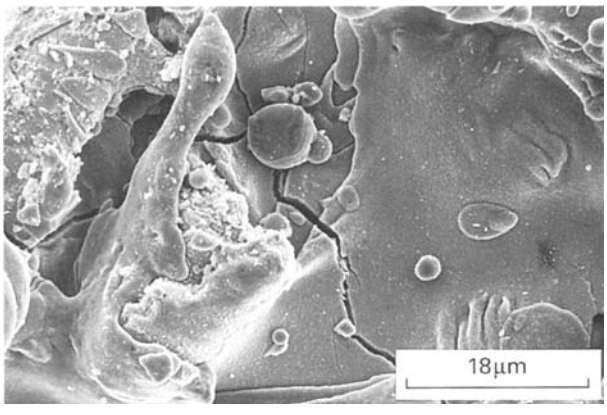

Figure 2 Scanning electron micrograph of Ti-PS surface confirming the roughness of Ti-PS surfaces.

TABLE II The real surface areas (RSA, in μm^2), as evaluated by CLSM, of the biomaterials investigated corresponding to 10014.1 μm^2 of apparent surface area (ASA)

Biomaterials	RSA (μm^2) ± SD	Percentage difference with respect to ASA
Ti-pol	10023.61 ± 5.65	+ 0.1
HA-pol	10116.85 ± 42.70	+ 0.3
Ti-PS	16068.51 ± 918.13	+ 60.5
HA-PS	14076.83 ± 45.95	+ 40.5

XRDC analysis showed that HA-pol samples were made up of 100% pure dense HA. The same technique revealed that the HA-PS samples were made up of HA (95%) and α-tri-calcium phosphate (α-TCP; 5% w/v). This bi-phasic composition was probably due to the HA-granules heating during plasma-spraying (Figs 5 and 6).

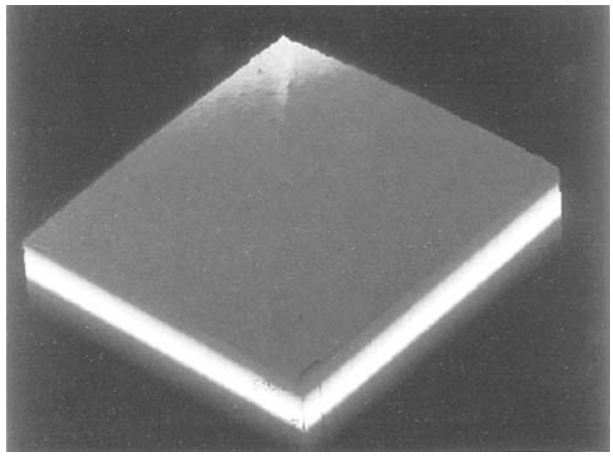

Figure 3 Surface topographical description of Ti-pol.

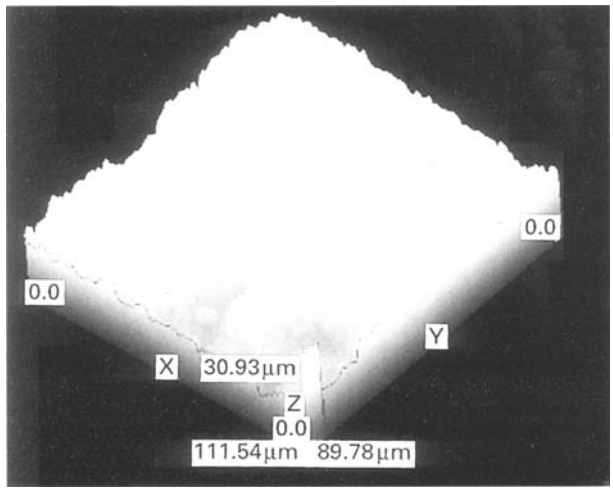

Figure 4 Surface topographical description of Ti-PS.

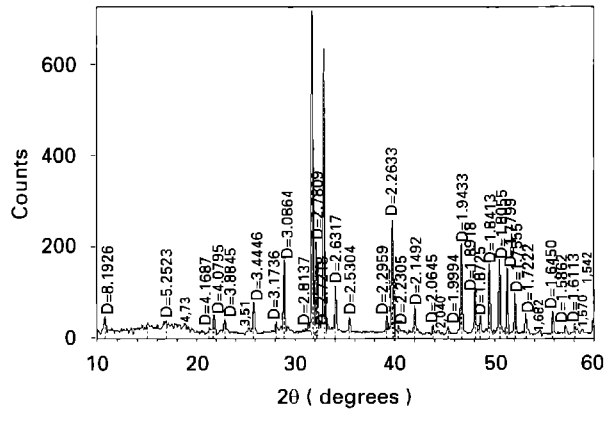

Figure 5 X-ray microanalysis of an HA-pol sample demonstrating a characteristic HA profile.

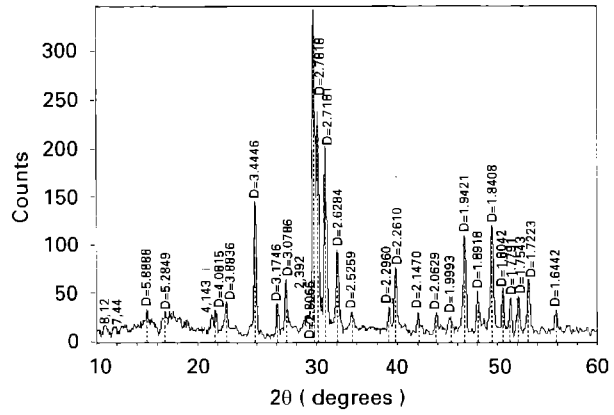

Figure 6 X-ray microanalysis of an HA-PS sample showing the presence of an HA phase greater than 95% combined with an α-TCP phase less than 5%. Peaks consistent with α-TCP are identified by □.

3.2. Adult human jaw bone cell cultures

Cells outgrown from bioptic jaw bone fragments (Fig. 7a) and propagated *in vitro* on standard plastic surfaces were found (i) to be able to produce and secrete type I collagen (Fig. 7b), (ii) to express a discrete basal ALP activity (Fig. 7c) that tended to increase when the cells were kept for extended periods *in vitro*, and (iii) to mineralize the collagenous matrix (Fig. 7d) when multi-layered cell cultures were grown in the presence of β-glycerophosphate. These morphofunctional findings indicated that cultured bone cells were capable of expressing the (pre)osteoblastic phenotype.

Therefore, we felt fully justified in growing these cells on Ti-pol, Ti-PS, HA-pol, and HA-PS discs and carrying out quantitative analyses of cellular activities, such as ALP activity, new DNA synthesis and osteocalcin secretion after 2 and 5 days of culture, and comparing these results with the corresponding activities of osteoblasts originating in the same patients cultured in parallel on Thermanox® coverslips. The results were corrected for the differences in RSA between the various materials tested (i.e. they refer to the same surface area whatever the material used), and hence are expressed in percentage change with respect to the activities exhibited on the second day *in vitro* by bone cells grown on Thermanox® coverslips.

De novo DNA synthesis. As shown in Fig. 8, human bone cells seeded onto Thermanox® coverslips exhibited active new DNA synthetic activity on day 2 *in vitro* that tended to increase further with time (at day 5, + 40% with respect to day 2; $p < 0.05$). From the data in Fig. 8 it is also clear that the HA-PS or HA-pol surfaces exerted a significant hindering action on bone cell DNA synthetic activity, with degrees of inhibition (as compared to cells grown on Thermanox®

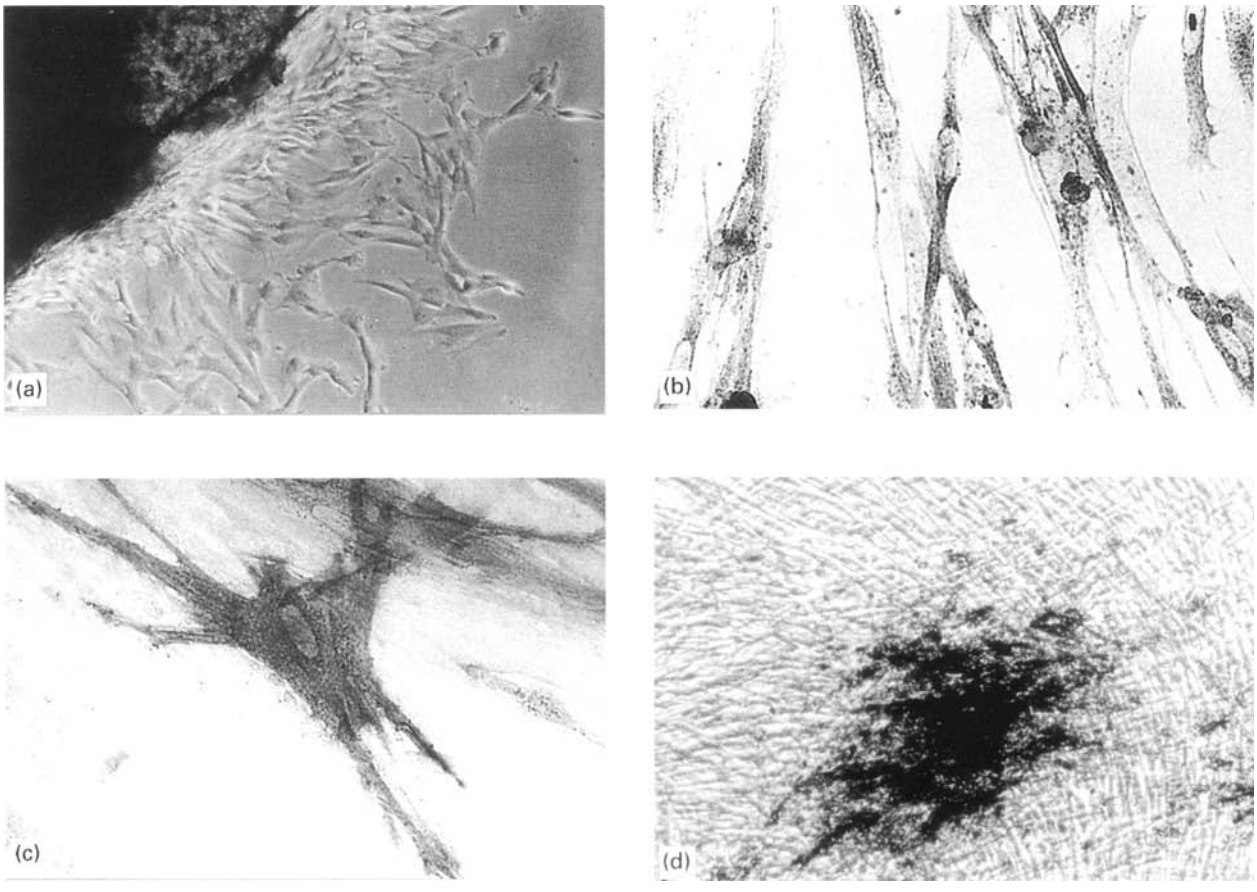

Figure 7 Adult human bone cell cultures. (a) Adult human osteoblasts migrate out of a jaw bone fragment during the first days of their time in primary culture. Phase contrast microscopy 100 ×. (b) An intensified intracellular presence of type I collagen characterizes maturing bone cells *in vitro*. Light microscopy, 100 ×. (c) Expression of ALP activity in a single human bone cell *in vitro*. Light microscopy, 320 ×. (d) Bone cells grow in multiple layers and form huge crystalline aggregates of insoluble calcium phosphate salts in the extracellular matrix in the presence of β-glycerophosphate. Von Kossa staining, light microscopy, 100 ×.

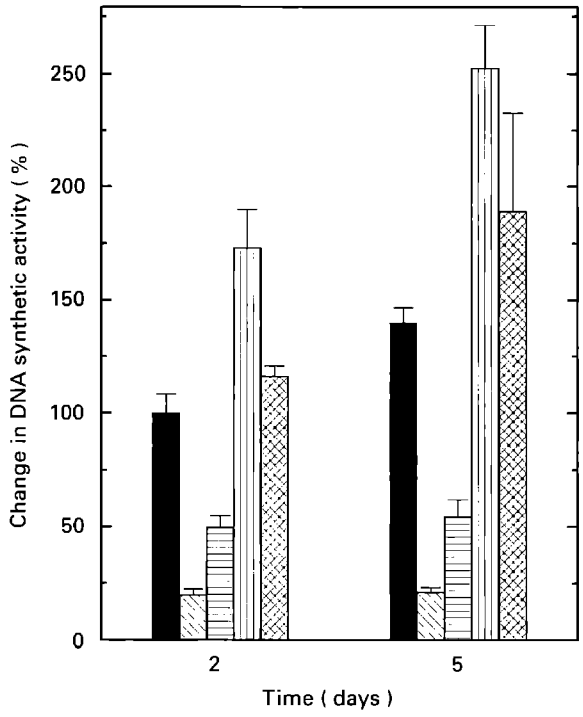

Figure 8 The *de novo* DNA synthetic activity of human jaw bone cells seeded onto different biomaterials and onto Thermanox® coverslips. [^{3}H] TdR was added to the culture media during the last 24 h preceding the samplings at days 2 and 5 *in vitro*. The values are means ± SEM of six distinct experiments and are expressed in arbitrary units as percentage changes of the cpm due to the macromolecularly incorporated [^{3}H] TdR/μg protein of bone cells grown for 2 days on Thermanox® coverslips, which were assumed to be equal to a value of 100. ■ Thermanox; ▨ HA-PS; ▤ HA-pol; ▥ Ti-pol; ▩ Ti-PS.

TABLE III Percentage changes in functional activities of human jaw osteoblasts cultured on the biomaterials tested with respect to the corresponding basal values of bone cells grown for 2 days on Thermanox® coverslips

Activity	Day 2	Day 5	Day 5 versus Day 2
DNA synthesis			
HA-pol	− 50[b]	− 46[b]	− 8.0
HA-PS	− 80[c]	− 79[c]	− 1.3
Ti-pol	+ 72[b]	+ 152[b]	+ 46.5
Ti-PS	+ 16[a]	+ 89[b]	+ 62.9
Cellular ALP activity			
HA-pol	+ 73[b]	+ 87[c]	+ 8.1
HA-PS	+ 77[b]	+ 82[b]	+ 2.8
Ti-pol	+ 89[b]	+ 176[c]	+ 46.0
Ti-PS	+ 29[a]	+ 129[c]	+ 77.5
Medium osteocalcin level			
HA-pol	+ 54[b]	+ 67[b]	+ 8.4
HA-PS	+ 117[b]	+ 117[b]	—
Ti-pol	+ 73[b]	+ 75[b]	+ 1.2
Ti-PS	+ 131[b]	+ 127[b]	− 1.7

The levels of significance (with respect to bone cells grown on Thermanox® coverslips) are as follows.[a] $p > 0.05$; [b] $p < 0.05$; [c] $p < 0.01$.

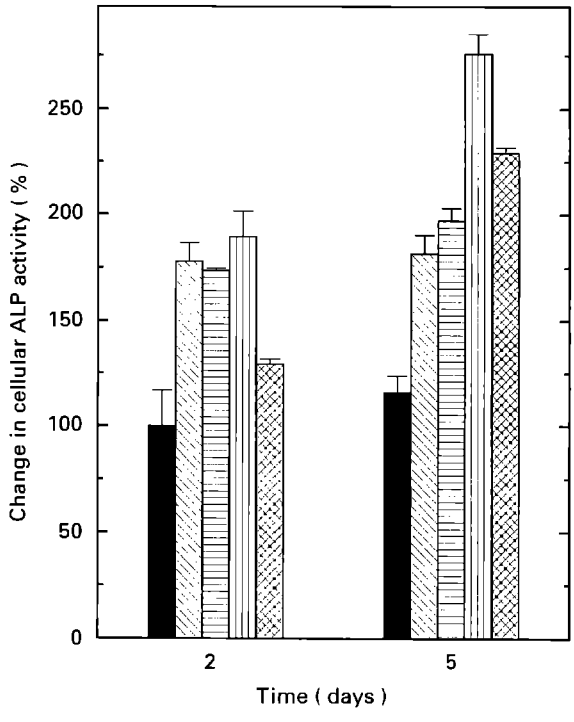

Figure 9 Levels of ALP activity in human jaw bone cells cultured on different biomaterials and on Thermanox® coverslips. At days 2 and 5 *in vitro*, the specimens were biochemically assayed for ALP activity. The values are means ± SEM of six distinct experiments and are expressed in arbitrary units as percentage changes of the U of ALP activity/mg of protein of bone cells grown for 2 days on Thermanox® coverslips, which were taken to be equivalent to a value of 100. ■ Thermanox; ▨ HA-PS; ▤ HA-pol; ▥ Ti-pol; ▩ Ti-PS.

coverslips) that did not change for either material between days 2 and 5 (Table III). In sharp contrast, bone cells grown on Ti-pol surfaces exhibited a remarkably more intense DNA synthetic activity than cells grown on any other kind of biomaterial (Fig. 8; Table III). However, bone cells grown on Ti-PS, though initially having a level of new DNA synthetic activity close to that of cells on Thermanox® coverslips (Fig. 8; Table III), exhibited the maximum increase (+ 62.9%) in DNA synthetic rate between day 2 and day 5. Hence, at variance with HA surfaces, Ti surfaces greatly favoured, both immediately (Ti-pol) and with time (Ti-pol and Ti-PS), the proliferation of human jaw bone cells in culture.

ALP activity. A discrete activity could be detected, both by histochemical and biochemical means, in human jaw bone cells kept for 2 days on Thermanox® coverslips (Fig. 9). This activity only slightly, but not significantly (+ 16%) increased between days 2 and 5 in these same specimens (Fig. 9). Cells grown for 2 days on HA-pol or HA-PS exhibited closely similar significant increases in ALP activity with respect to cells on Thermanox® coverslips (Fig. 9; Table III). Such increases had changed very little (+ 2.8 − + 8.1% with respect to day 2 levels) after 5 days of culture (Fig. 9; Table III). Hence, HA surfaces of either kind had an immediate, steady, and long-lasting positive effect (with respect to the Thermanox® surface) on ALP activity in human bone cells. Similarly, on day 2, cells cultured on Ti-pol discs exhibited an ALP activity only slightly greater than that of cells grown on HA surfaces (Fig. 9; Table III). However, a discrete increase in ALP activity (+ 46.0% with respect to day 2 levels) could be detected in cells cultured for 5 days on Ti-pol surfaces (Fig. 9;

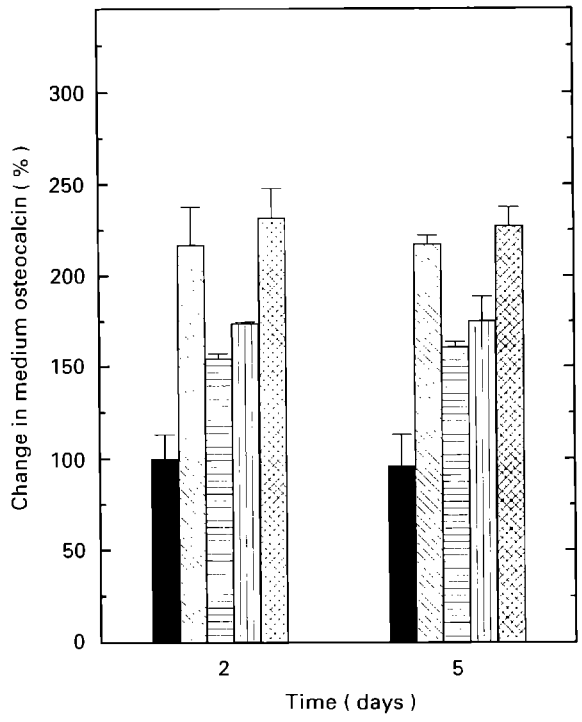

Figure 10 The levels of osteocalcin secreted into serumless growth medium samples conditioned by jaw bone cells cultured on different biomaterials and on Thermanox® coverslips. The specimens represent the osteocalcin secreted during the last 48 h prior to the samplings at days 2 and 5 *in vitro*. The values are means ± SEM of six distinct experiments and are expressed in arbitrary units as percentage changes of the ng osteocalcin/mg of cell proteins in the corresponding cultures of bone cells grown for 2 days on Thermanox® coverslips, which were taken to be equivalent to a value of 100. ■ Thermanox; ☐ HA-PS; ▤ HA-pol; ▥ Ti-pol; ▦ Ti-PS.

Table III). Finally, bone cells grown on Ti-PS surfaces had, by day 2, an ALP activity only slightly higher (+ 29%) than that of cells seeded on the Thermanox® discs, and hence lower than that of bone cells kept on HA-pol, HA-PS, and Ti-pol surfaces (Fig. 9. Table III). However, the most remarkable rate of increase in ALP activity (+ 77.5% with respect to day 2 levels) was detected in bone cells cultured for 5 days on Ti-PS discs (Fig. 9; Table III). Hence, Ti-pol surfaces exerted an immediate and persistent effect on ALP activity in human jaw bone cells, whereas Ti-PS surfaces had a somewhat weaker initial positive effect that was compensated by a later very fast rate of increase with time in culture.

Osteocalcin secretion into the growth medium. Jaw bone cells cultured on the Thermanox® surface exhibited, during the first 2 days, a discrete level of osteocalcin secreted into the culture medium, which changed little (− 4%) between days 4 and 5 (Fig. 10). The levels of osteocalcin secreted during days 1 and 2 were found to be significantly and similarly higher in cultures seeded onto HA-PS and Ti-PS discs, and to remain practically steady during days 4 and 5 (changes from 0 to − 1.74% with respect to previous levels) (Fig. 10; Table III). Hence, both kinds of plasma-sprayed surfaces exerted similarly enhancing, steady, and long-lasting effects (with respect to the Thermanox® surface) on the osteocalcin secretion on the part of jaw bone cells in culture. On the other hand, both types of polished surfaces had similarly persistent but much less intense effects on the secretion of osteocalcin into the growth medium (Fig. 10; Table III).

4. Discussion

The present results show that a proper correlation of the physical properties of various kinds of biomaterials with the expression of specific cellular differentiated functions can greatly contribute to a deeper comprehension of their suitability for clinical applications.

It is clear that both types of HA surfaces tested hindered, though with different degrees of effectiveness, new DNA synthetic activity of human bone cells cultured on them, while allowing significant amounts of ALP activity and of osteocalcin secretion to simultaneously occur. Moreover, it is also important to note that the levels of the various activities expressed by the bone cells grown on either type of HA surfaces did not significantly change between day 2 and day 5 of culture (Table III). Taken collectively, these findings appear to imply that HA surfaces, either polished or plasma-sprayed, markedly prevented by a presently unknown mechanism the proliferation of the relatively undifferentiated pre-osteoblasts precursors, while simultaneously allowing the proper functioning of the pre-osteoblasts and the osteoblasts already present in the cultures. In fact, it should be recalled here that ALP, a plasmalemmal enzyme, is expressed by both pre-osteoblasts and osteoblasts, whereas osteocalcin secretion is a specific marker of the active osteoblasts [15]. However, since osteocalcin secretion was found to be much more intense when human bone cells were cultured on HA-PS than on HA-pol surfaces, it seems conceivable that the rough HA surface favoured a more intense exocytotic activity on the part of a non-expanding population of osteoblasts, which probably at least in part survived in tri-dimensional cultures in the depths of the HA-PS valleys. Reportedly, tri-dimensional cultures allow a much more intense expression of specific differentiated functions than bi-dimensional cultures (such as those on HA-pol surfaces) do [16].

On the other hand, our present findings clearly reveal that the proliferative activity of human bone cells was intensely, immediately, and persistently enhanced by carrying out the cultures on Ti-pol surfaces, whereas bone cells grown on Ti-PS surfaces remarkably increased their growth-related activities only after a certain delay. Bone cells cultured on Ti-pol surfaces also exhibited high levels of ALP activity that increased further with time *in vitro*. Conversely, bone cells grown on Ti-PS surfaces initially possessed Thermanox® -like levels of ALP activity, which, between day 2 and day 5 *in vitro*, exhibited rates of increase that were higher than those shown in cells grown on Ti-pol surfaces. Finally, osteocalcin secretion was much more intense in Ti-PS cultures than in Ti-pol cultures, thus repeating the picture observed for the corresponding HA cultures. On the whole, these findings are consistent with the view that, at variance with the hyper-differentiating HA surfaces, both kinds of Ti

surfaces tested can enhance the proliferation of pre-osteoblastic precursors, Ti-PS showing maximum effectiveness after a short delay. As one can gather from the observed levels of ALP activity, this growth-favouring effect on pre-osteoblastic precursors elicited by both kinds of Ti surfaces is mirrored by the significant increases, detectable after 5 days *in vitro*, in the sizes of the pre-osteoblastic populations grown on either kind of Ti discs, with Ti-PS having the maximum time-related rate of increase in ALP activity. On the other hand, the data collected on the levels of osteocalcin secreted into the growth medium indicate that the numbers of osteoblasts grown on either type of Ti surfaces did not significantly change within the first 5 days of culture. However, as discussed above for HA-PS surfaces, the Ti-PS material greatly favoured the secretion of osteocalcin, probably by also allowing the establishment of tri-dimensional cultures in the depth of its canyons.

5. Conclusions

It is stressed here that this is a preliminary investigation, in which the levels of the functional activities of cultured adult human bone cells from jaw bones were for the first time corrected for the RSA of the biomaterials on which they were grown to attain a more precise evaluation of the interactions occurring between bone cells and biomaterials. Furthermore, it seems likely that the maximum time investigated (5 days) is too short to allow the observation of significant changes in the size of the osteoblastic populations grown on the various biomaterials. Reportedly, osteoblasts require time lags somewhat longer than 5 days to reach full maturation in tissue culture settings [10]. Notwithstanding this, from the standpoint of clinical application, it appears that a biomaterial allowing for significant expansion of the pre-osteoblastic precursors seeded onto it would be more advantageous than a biomaterial that nearly totally hinders cell proliferation. Hence, it would be reasonable, when considering implantological applications of cultured human bone cells, to select Ti surfaces rather than HA surfaces. As to what kind of Ti surface should be preferred, our findings indicate that on a very short-term basis, Ti-pol surfaces have some advantage. However, it should be stressed that on a longer-term basis Ti-PS surfaces exhibit the maximum time-related rates of increase in both DNA synthetic and ALP activities. Hence, Ti-PS surfaces are likely to be better suited to clinical applications, as further longer-lasting investigations, already under course, will probably show.

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