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Attachment kinetics, proliferation rates and vinculin assembly of bovine osteoblasts cultured on different pre-coated artificial substrates

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Primary bovine osteoblasts were used to study *in-vitro* effects of attachment on vinculin assembly in cells cultured on various artificial substrates. Materials coated with fibronectin and bovine serum albumin (BSA) as well as untreated materials (tissue culture polystyrene and Aclar foils) were chosen to investigate substrate-dependent proliferation during the first 3 days of culture. Proliferation was highest on fibronectin-coated substrates, followed by BSA-coated and untreated substrates. During the first 24 h of cultivation, cell attachment kinetics revealed no significant difference between the various substrates. After 24 h detachment rates obtained by calcium depletion with ethylenediaminetetraacetic acid were highest on uncoated materials, followed by BSA- and fibronectin-coated substrates. Phase contrast microscopy revealed typical osteoblast morphology after cell adhesion for 24 h. The dynamic attachment process was concomitant with the reassembly of vinculin into streak-like focal contacts clustered on the ventral side of cells. The kinetics of vinculin reassembly were independent of the underlying coating. Thus, fibronectin coating of artificial substrates increased the attachment strength and proliferation rate of osteoblasts. While the reassembly of vinculin in focal contacts seems to be a prerequisite of osteoblast attachment *in vitro*, it does not seem to have profound effects on the subsequent cell behaviour on artificial substrates.

1. Introduction

With most orthopaedic and dental prostheses a strong, durable and stable adhesion of bone to an implant is desirable. The nature of the bone–implant interface is determined by many factors including cellular responses to the implant surface. An ideal implant should exhibit intimate apposition of bone tissue to the implant surface. Therefore knowledge of the responses of bone-forming osteoblasts to synthetic implant surfaces could aid in the assessment of implant performance.

Protein adsorption is believed to be the first event that takes place after contact of body fluids with artificial surfaces [1]. Subsequently the protein-covered artificial substrates initiate the adhesion of cells. The attachment of anchorage-dependent cells to synthetic surfaces is a prerequisite to subsequent transcriptional and translational events [2–6]. In bone tissue the predominant cell type interacting with artificial substrates is the osteoblast. The interaction of these cells with surfaces has profound effects on many important biological processes, including adhesion, proliferation, migration and phenotypic differentiation [2, 4–12].

Cells attach to substrates through distinct contact sites which are classified depending on the distance of the cell from the substrate and the presence of certain adhesion proteins as focal contacts, close contacts and extracellular matrix contacts [13]. Focal contacts are tenacious adhesion sites that remain attached to the substratum even when cells are forcibly detached, indicating their function as anchorage structures [13–15]. Vinculin, a 130 kDa cytoskeletal protein localized in focal contacts, has been proposed to be involved in the attachment of microfilaments to the plasma membrane [5, 14–18]. Although vinculin is usually noted for its presence in focal contacts, it is also present in a soluble cytoplasmic pool [15, 17].

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From a biomaterial perspective, the nature of the contact sites formed between cells and surfaces may influence tissue reactions to substrates. Since these responses may subsequently affect the fate of bone implants, it is important to understand the nature of contact site formation by osteoblasts interacting with different pre-coated biomaterials. Our aim in the presented study was to elucidate the attachment and growth characteristics of periosteal derived osteoblasts during short-term cultivation on different pre-coated substrates. Thus, we have investigated cell attachment kinetics as well as proliferation rates of osteoblasts cultured on various protein-coated and uncoated synthetic substrates under *in vitro* conditions.

2. Materials and methods

2.1. Materials

Three different artificial substrates were evaluated. Tissue culture polystyrene (Nunclon, Wiesbaden, Germany), Aclar foils (Allied Chemical, Clifton, NJ, USA) and borosilicate glass were chosen, since they provide good substrates for cell attachment and growth. All cell culture experiments were performed under sterile conditions. The materials with a smooth glass-like surface were coated with fibronectin or bovine serum albumin (BSA) before cells were seeded. For fibronectin coating we used bovine fibronectin ($15\ \mu g\,ml^{-1}$) (Serva, Heidelberg, Germany) diluted in phosphate-buffered saline (PBS) (8 g of NaCl, 0.2 g of KCl, 2.16 g of $Na_2HPO_4 \times 7H_2O$, 0.2 g of $KH_2PO_4\,l^{-1}$) and dispersed into multiwell chambers ($5\ \mu g\,cm^{-2}$). After incubation for 24 h at 37 °C all wells were soaked three times with PBS. Non-specific binding sites were then blocked by incubation with 0.1% BSA in PBS for 1 h followed by two washing steps with PBS. BSA (Serva, Heidelberg, Germany) coating was obtained by incubating the different surfaces with 0.1% BSA in PBS for 24 h at 37 °C. Substrates were finally washed twice with PBS and prepared for cell seeding. Prior to cell seeding, PBS was removed and the substrates were washed once with Ham's F10 medium.

2.2. Cell culture

Cells were prepared using the outgrowth method previously described by Jones *et al.* [19]. Tissue specimens from metacarpals of 18 month old steers were obtained at the local slaughterhouse. The metacarpals were dissected free of muscular tissue under sterile conditions. Periosteum pieces were removed and cultured in High-Growth-Enhancement Medium (Flow Laboratories, Rickmansworth, UK) containing 10% foetal calf serum (FCS) for 4–5 weeks. The culture medium was changed once a week. The outgrowth of cells led to a homogeneous culture of osteoblast-like cells. Cells were characterized by the presence of osteocalcin and by the detection of high alkaline phosphatase activity. After confluence the cells were harvested by collagenase incubation (0.4 g of collagenase and 98.8 mg of Ham's F10 in 10 ml of 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid buffer) for 20 min followed by treatment with Tyrode's solution (300 mg of ethylenediaminetetraacetic acid (EDTA)-Na salt, 200 mg of KCl, 8 g of NaCl, 1 g of $NaHCO_3$, 50 mg of NaH_2PO_4 and 1000 mg glucose l^{-1}). Cell numbers were counted in a Coulter counter. To determine the *in vitro* appearance of osteoblasts during different stages of attachment, micrographs were taken with a Zeiss phase contrast microscope. Osteoblasts grown for 24 h on glass coverslips were exposed to Tyrode's solutions with different EDTA concentrations (0%, 0.005%, 0.01% and 0.03%, respectively). After 20 min incubation with the different EDTA concentrations, cells were fixed with cold methanol (−20 °C for 6 min) and processed for indirect immunofluorescence assay.

2.3. Antibodies

For immunofluorescence staining antivinculin antibodies were diluted to 1:400 in 2% BSA in Tris-buffered saline (TBS) (20 mM Tris-HCl and 150 mM NaCl; pH 7.4). The monoclonal antivinculin antibody from clone hVIN purified from ascites fluid was obtained from Sigma, Deisenhofen, Germany. As secondary antibody fluorescein isothiocyanate (FITC)-labelled rabbit antimouse immunoglobulins (IgGs) purchased from Dakopatts, Glostrup, Denmark were used at concentrations of 1:50 diluted in 2% BSA–TBS.

2.4. Plating efficiency

The plating efficiency was defined as the percentage of substratum-attached cells 24 h after seeding. Preliminary experiments established the plating efficiency for each surface, so that distinct cell numbers were used to compare attachment kinetics and growth on the various surfaces. For attachment experiments the same number of viable cells were used as determined by the Trypan Blue exclusion test. Briefly, different materials were attached to multiwell chambers (diameter, 1 cm). 500 µl of medium (Ham's F10 + 10% FCS) containing 6×10^4 viable cells cm^{-2} were added to each chamber to give a subtotal confluency. Cells were incubated at 37 °C in an atmosphere of 5% CO_2. The supernatants were removed after 1, 4, 7 and 24 h, respectively, and cell numbers of suspended cells were exactly the inverse of the numbers of attached cells. The aliquots each from four chambers were counted four times. The results were expressed as the mean of the cell count with standard deviations ($n = 4$ and 32 measurements).

2.5. Rate of cell detachment

Detachment experiments were performed 24 h after cell seeding under the same conditions as described above except that 8.5×10^4 viable cells cm^{-2} were used per chamber. Cells attached to the surface were treated by incubation with Ca^{2+}- and Mg^{2+}-free Tyrode's solution containing 0.03% EDTA for 20 min. Aliquots from the supernatants containing detached cells were counted in a Coulter counter. The

surfaces were then viewed under a microscope equipped with Nomarski differential contrast optics.

2.6. Cell proliferation assay

To determine the proliferation of osteoblast-like cells on the different substrates, osteoblasts were plated in Ham's F10 medium at a density of 4.0×10^4 cells cm^{-2}. Cells were cultured in 70 well plates on pure polystyrene and Aclar matrices as well as on fibronectin- and BSA-coated substrates. The medium was not changed during the culture time. Cells were harvested after culture times of 1, 2 and 3 days, respectively. Collagenase incubation for 20 min followed by Tyrode's solution with a final pronase treatment (0.5 mg pronase (ml Tyrode's solution)$^{-1}$) was performed as described above, until all adherent cells were detached. Cell numbers were counted in a Coulter counter and the results were expressed as means with standard deviations.

2.7. Indirect immunofluorescence microscopy

Indirect immunofluorescence microscopy was performed at different stages of cell adhesion. After cell seeding for 1 h, 4 h, 24 h and 3 days on the different pre-coated substrates, the medium was removed and cells were extracted with 0.25% Triton X-100 in 140 mM NaCl, 5 mM EDTA, 25 μM 2-mercaptoethanol and 10 mM Tris-HCl (pH 7.6) for 45 s at room temperature. Osteoblasts were fixed in methanol at −20 °C for 6 min after a washing step in PBS. Incubation with both primary and secondary antibodies was for 45 min at 37 °C with three washes in PBS between each incubation. Stained specimens were photographed with a Zeiss Axiophot photomicroscope on Kodak Tri-X-Pan 400 films after mounting coverslips in Mowiol 4-88 (Hoechst, Frankfurt am Main, Germany). For the detection of cytoplasmic vinculin, cells were fixed in methanol without prior incubation with Triton X-100.

3. Results

The attachment kinetics of osteoblast cells on distinct artificial substrates were studied with a single cell suspension prepared by trypsinization of a stock culture. Fig. 1a and b shows the results of our plating efficiency experiments with bovine osteoblasts on uncoated and coated polystyrene and Aclar surfaces, respectively. During the first 4 h after seeding, a steep decrease in the cell number of the supernatant was measured for all surfaces. As expected, the loss of cells in the supernatants was exactly paralleled by an increase in the number of cells attached to the surfaces (data not shown). The rate of cell adhesion was slightly higher on BSA-coated surfaces, followed by fibronectin-coated and uncoated surfaces. There was no significant difference between the two tested materials (polystyrene versus Aclar). Between 7 and 24 h the attachment rate remained stable on all coatings and underlying substrates. After 24 h the number of non-adherent cells on all surfaces was below 10% of the initial cell number.

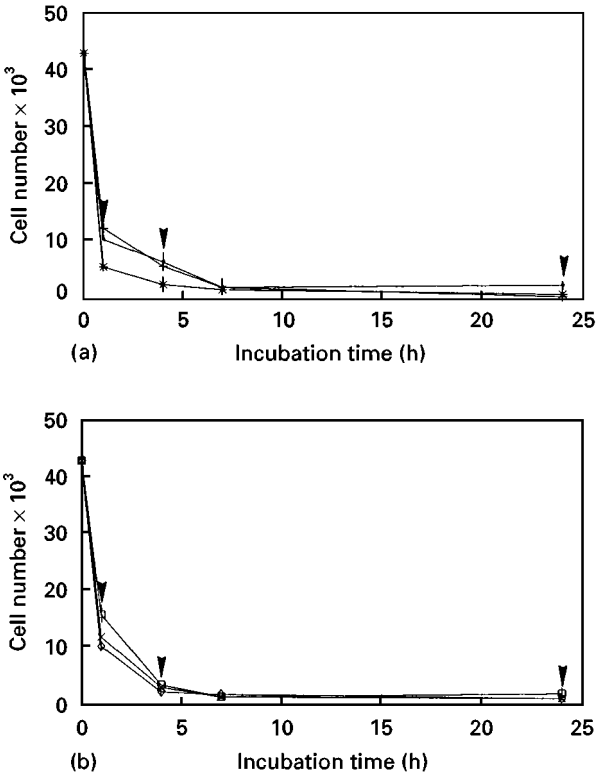

Figure 1 Plating efficiency of bovine osteoblasts on protein-coated and uncoated tissue culture (a) polystyrene and (b) Aclar surfaces. (●), polystyrene; (+) fibronectin-coated polystyrene; ⋆, BSA-coated polystyrene; (□), Aclar; (×), fibronectin-coated Aclar; (◇), BSA-coated Aclar. Absolute cell numbers of non-adherent cells in the supernatants were measured after incubation for 1, 4, 7 and 24 h. Prior to cell seeding, materials were coated with fibronectin and BSA. Data are mean values of four individual determinations (± standard deviation).

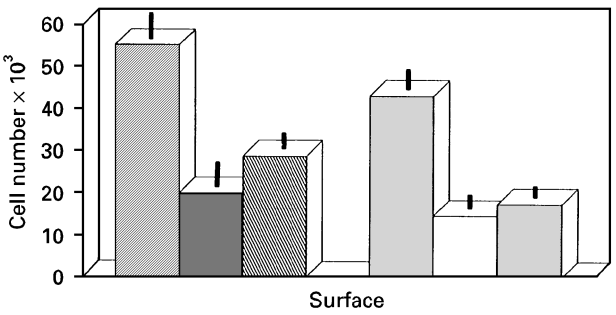

Figure 2 Cell detachment rates of adherent osteoblasts on fibronectin- and BSA-coated and uncoated substrates (polystyrene and Aclar) 24 h after seeding. The columns indicate the number of detached cells by a standard EDTA treatment (initial cell number, 85 000) and from left to right represent polystyrene, fibronectin-coated polystyrene, BSA-coated polystyrene, Aclar, fibronectin-coated Aclar and BSA-coated Aclar. The data are mean values of four determinations with standard deviations.

Detachment rates obtained by EDTA treatment 24 h after cell seeding revealed significant differences between uncoated and protein-coated materials (Fig. 2). EDTA treatment detached the majority of cells from the untreated surfaces, whereas a large number of cells remained on the surface of protein-covered materials. Fibronectin coating resulted in a high number of adherent cells after EDTA treatment on both

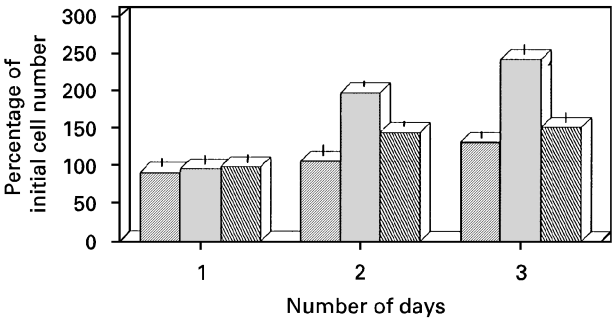

Figure 3 Proliferation rates of osteoblasts cultured on uncoated polystyrene (▨) and fibronectin-coated polystyrene (▤) and BSA coated polystyrene (▧) surfaces during the first 3 days after cell seeding. The columns indicate the percentage of cells on the various surfaces compared with the initial cell number set as 100%. Data are presented as mean values of four determinations with standard deviations.

materials (75% ± 6% on polystyrene and 82% ± 3% on Aclar). On BSA-coated substrates the number of cells remaining attached was slightly lower (66% ± 4% on polystyrene and 80% ± 3% on Aclar). The rate of attached cells was significantly higher on Aclar than on polystyrene surfaces. By an additional pronase treatment step all adherent cells were detached.

Cell growth during the first 3 days differed between the various surfaces studied. Fibronectin coating of polystyrene exhibited the best growth stimulation, followed by the BSA-coated and uncoated polystyrene surfaces (Fig. 3). After 3 days the cell number on fibronectin-coated materials was increased approximately 2.5-fold, while on untreated polystyrene surfaces the cell number increased only about 50%. The proliferation rate on BSA-coated substrates was in between that of fibronectin-coated and uncoated surfaces. Cell growth on the uncoated and coated Aclar foils was comparable with growth on polystyrene surfaces (data not shown).

The morphology of osteoblast-like cells in various stages of cell adhesion was observed in phase contrast microscopy (Fig. 4). In the initial stage 1 h after cell seeding, osteoblasts attached loosely to the surface, acquiring a spheroidal shape (Fig. 4a). Prominent nuclei were predominantly localized in the periphery of the attachment area between the cell and the artificial substrate. In a subsequent stage, osteoblasts spread on the surface, resulting in a flat monolayer after 4 h (Fig. 4b). Multiple liposomes surrounded the centrally retracted nucleus. After 24 h, in a later stage of cell surface interaction, osteoblasts appeared to have intimate contact with the substrates, exhibiting their typical polygonal morphology (Fig. 4c). Protein coating seemed not to influence the gross appearance of osteoblast-like cells in later stages, since phase contrast microscopy revealed no difference between the various protein-coated materials.

To observe the assembly of vinculin during cell–surface interaction, indirect immunofluorescence microscopy was performed. Methanol fixation was used for the detection of vinculin in osteoblasts on the various substrates studied. During the initial stage of

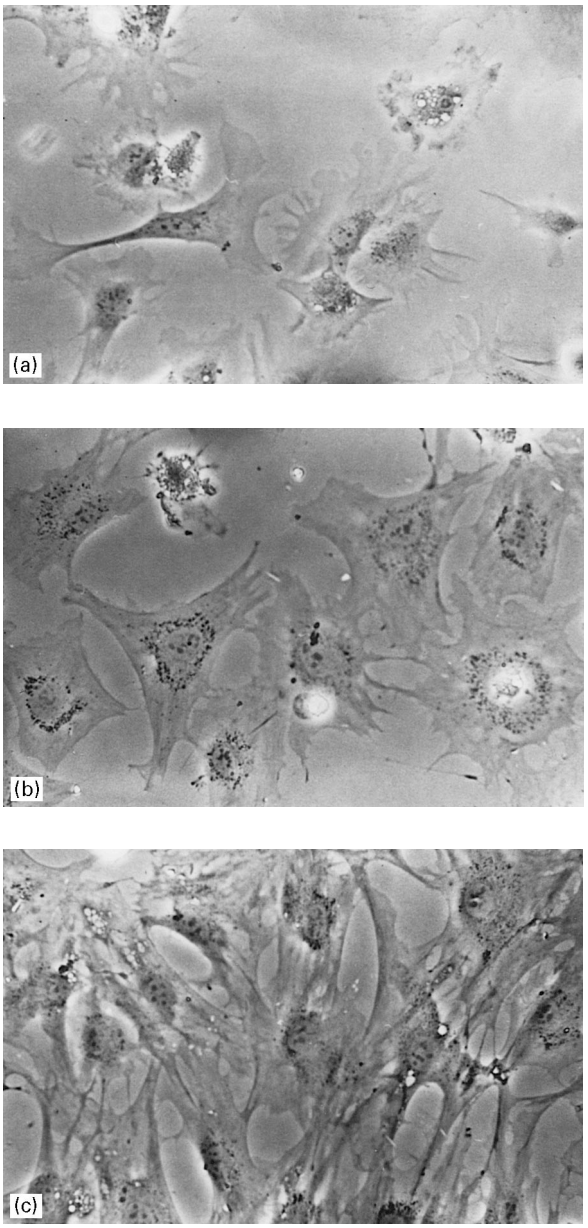

Figure 4 Morphology of osteoblasts during the attachment on polystyrene. Phase contrast microscopy shows the typical appearance of osteoblasts after culture for (a) 1 h, (b) 4 h and (c) 1 day.

cell adhesion, vinculin-containing focal contacts were found predominantly at the cell periphery in a punctate pattern (Fig. 5a). As the cells flattened on the surfaces, vinculin-containing focal contacts were clustered in cellular extensions in an apparent attempt to spread their cell bodies over the substrates (Fig. 5b). In the final stage, vinculin-containing focal contacts were scattered throughout the whole flattened cellular area (Fig. 5c), leading to intimate contact between the surface and the cell. After methanol fixation, osteoblasts exhibited a diffuse cytoplasmic immunofluorescence background staining at the different stages of cell adhesion.

Cell lysis by Triton X-100 prior to methanol fixation was performed to remove this cytoplasmic fraction. Fig. 6a shows osteoblasts cultured for 24 h on borosilicate glass. Vinculin-containing adhesion plaques were found in a discrete streak-like pattern at the cell periphery. Cell lysis by Triton X-100 led to an

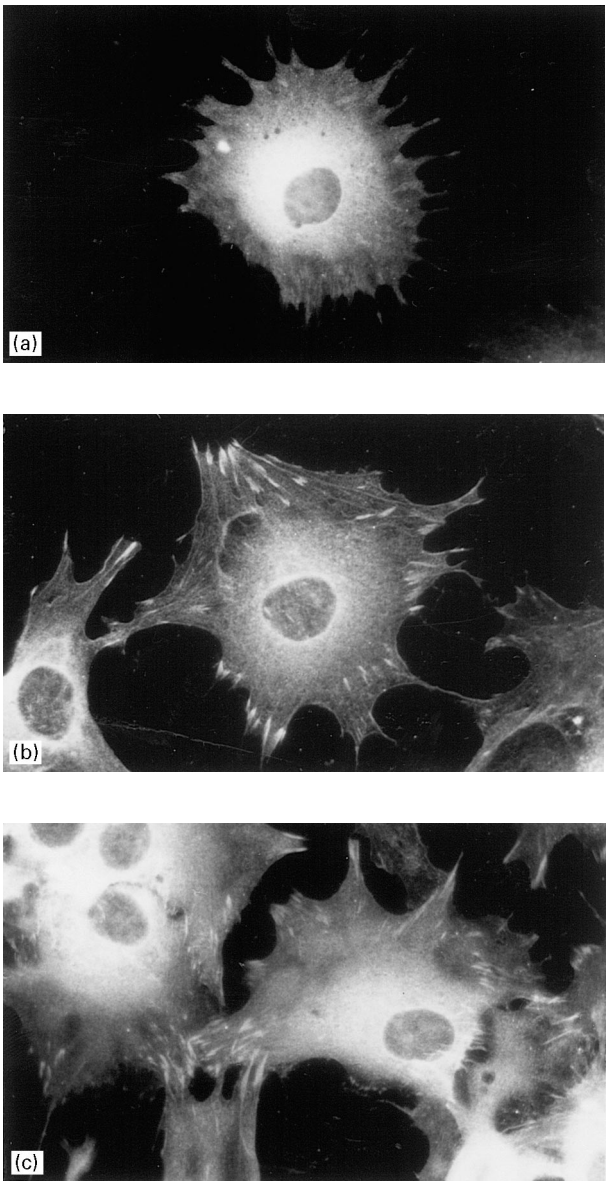

Figure 5 Localization of vinculin in osteoblasts during adhesion and growth on borosilicate glass. Cells were labelled with monoclonal vinculin antibody hVIN-1 as primary and FITC-labelled rabbit anti-mouse IgGs as secondary antibody. (a) At an initial attachment stage (1 h) osteoblasts displayed vinculin in a punctate circular pattern at their cell margins. (b) After 24 h, multiple vinculin containing focal contacts were visible as streak-like deposits predominantly at the periphery. (c) Vinculin was localized in multiple patches scattered throughout the ventral cellular area after culture for 3 days.

intensive staining of clustered vinculin and abolished nearly all background staining.

To reveal the effect of calcium depletion on vinculin assembly, osteoblasts were incubated for 20 min in a Ca^{2+}-free Tyrode's solution containing different EDTA concentrations. At EDTA concentrations above 0.005% a disruption of adhesion integrity was observed. The cell detachment was paralleled by a redistribution of vinculin from streak-like focal contacts to a homogeneous cytoplasmic localization (Fig. 6b). Cells gained a spheroidal morphology as seen during the initial stage of cell attachment with long filopodia-like cell extensions. Despite the different detachment rates of osteoblasts on the various substrates studied, no gross difference in the pattern of vinculin

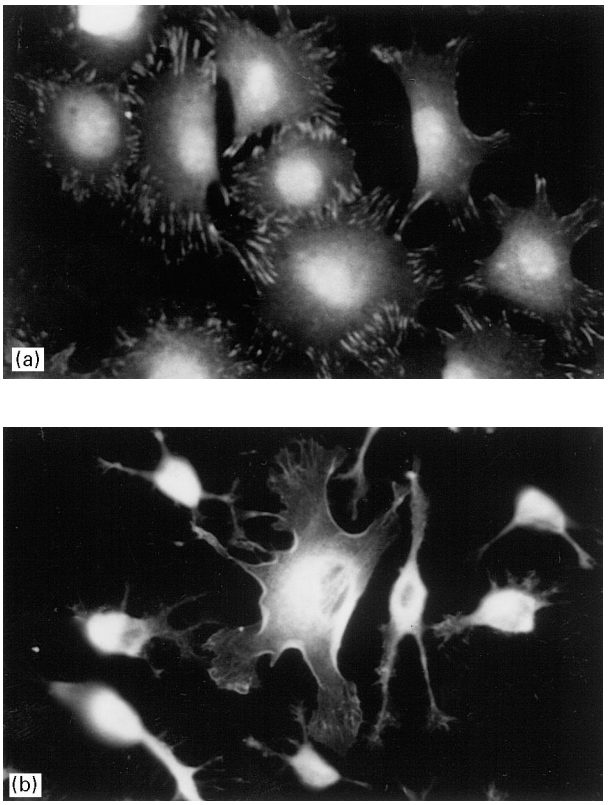

Figure 6 Effect of tritonization and calcium depletion on vinculin staining in immunofluorescence. (a) In cytoskeletons of cultured osteoblasts obtained by Triton X-100 treatment (60 s; 0.1% Triton), immunostaining revealed vinculin preferentially in focal contacts. (b) Calcium depletion by incubation with 0.005% EDTA for 20 min demonstrates the disruption of adhesion plaques, resulting in the release of vinculin from focal contacts.

reassembly between protein-coated and uncoated materials was found.

4. Discussion

A major challenge for recent orthopaedic and dental surgery is to produce functional prosthetic bone grafts for use in patients. Although factors initiating bone differentiation are poorly understood, surface properties seem to be of crucial importance for osteogenesis [4, 6–12]. Successful adhesion and spreading on artificial surfaces are prerequisites for inducing new bone formation locally at the site of implantation. Therefore reliable *in-vitro* tests for cell adhesivity on potential prosthetic implant surfaces would be helpful in predicting their capability as biomaterials. Protein pre-coating of denuded implant surfaces may influence the biocompatibility of implant materials so as to support and induce osteogenesis. In this respect, cell culture models offer the possibility of evaluating different effects of biomaterials on a variety of cellular responses including attachment, proliferation, migration and differentiation. Thus, we focused our interest on the adhesion process of osteoblasts on artificial surfaces pre-coated with fibronectin or BSA.

Cell adhesion is a complex process depending on multiple interactions between surface topography, matrix receptors such as integrins and diverse cytoplasmic proteins [2, 8, 20–24]. Several stages of

osteoblast adhesion to substrates can be distinguished. The initial attachment is followed by the formation of focal adhesion sites and subsequent cell spreading [10, 16]. It has been suggested that focal adhesion to extracellular matrix proteins alters gene expression and leads to changes in the cytoskeleton [2, 3, 13, 23]. Phosphorylation of signalling proteins by cytoskeleton-associated protein kinases may induce structural alterations responsible for subsequent morphological changes [5, 14, 17, 25, 26].

In our study we investigated the attachment properties of osteoblasts adherent to protein-coated and uncoated substrates. As judged by the expression of osteocalcin and alkaline phosphatase, periostal-derived cell lines from bovine metacarpals subsequently adopted morphological features characteristic of cultured osteoblast-like cells. We showed that attachment kinetics appeared to be similar on both polystyrene surfaces and Aclar foils. These findings, when compared with previous investigations of cell attachment kinetics of various biomaterials with different wettabilities, suggest that both materials tested exhibited similar attachment properties due to their similar wettabilities [8, 12]. Our results are comparable with those found by others, demonstrating that attachment of osteoblasts differed between protein-coated and uncoated polystyrene surfaces during the first hours of incubation, while reaching a stable plateau phase after 24 h [4, 8, 12].

Detachment rates of osteoblasts obtained by treatment with EDTA revealed significant differences between the various surfaces. On fibronectin-coated surfaces, most cells remained on the substrate ($75\% \pm 6\%$ on polystyrene; $82\% \pm 3\%$ on Aclar), whereas on pure polystyrene and Aclar foils the number of detached cells was increased ($34\% \pm 4\%$ and $47\% \pm 3\%$, respectively). The higher adhesion rate on fibronectin-coated substrates than on BSA-coated substrates may be due to a specific binding between fibronectin receptors on osteoblasts and their extracellular ligands. Recent studies indicate that, despite the known calcium-dependent binding of fibronectin to the fibronectin receptor, cell attachment to fibronectin and superfibronectin is only partially inhibited by EDTA treatment [27]. From several other studies it is known that receptor blocking by specific antibodies recognizing the RGD sequence (Arg–Gly–Asp) in matrix proteins inhibits cell adhesion [22–24]. Our findings in normal non-transformed bone cells are in line with previous results examining the fibronectin-receptor-dependent adhesion in human osteosarcoma cells and primary human bone cells [7, 28, 29].

Here we show that the different cell adhesion properties of pure materials and protein-coated substrates were associated with significant differences in the growth rate of osteoblasts. Fibronectin coating of tissue culture polystyrene stimulated cell growth, whereas on pure polystyrene surfaces a much lower proliferation rate was measured. In our study there was no significant difference in vinculin assembly during attachment of osteoblasts on the various underlying substrates (polystyrene, Aclar and borosilicate glass). The attachment of osteoblasts on artificial surfaces was paralleled by the formation of vinculin-containing focal patches [11]. In early stages of attachment, vinculin was organized predominantly at the cell periphery in a fine punctate circular pattern. After 4 h, flattened osteoblasts assemble vinculin in more or less streak-like deposits. In later stages, after 24 h, vinculin-containing focal contacts were observed scattered throughout the ventral area of the cell.

The assembly of vinculin in focal contacts in various cell lines has been demonstrated in immunofluorescence studies [2, 11, 16, 30]. By using methanol fixation instead of the widely performed formaldehyde fixation, we showed the presence of vinculin in focal contacts as well as in the cytoplasm. The use of transparent materials allowed us to differentiate between a fibrillar and a homogeneous cytoplasmic arrangement of vinculin. Immunofluorescence staining obtained after cell lysis with Triton-X labelled only vinculin clustered in focal contacts but not the diffuse cytoplasmic pool. The low background stain indicated that tritonization solubilizes most of the vinculin found on the cytoplasmic pool.

Calcium depletion by EDTA treatment led to a dramatic redistribution of vinculin from focal contacts into a diffuse cytoplasmic pattern. The disassembly of focal contacts was paralleled by a change in cell morphology. At low EDTA concentrations (0.005%), osteoblasts retracted their cell bodies and exhibited a more spheroidal shape with long filopodia-like structures remaining on the substrate. The dynamic detachment process is concomitant with the disassembly of vinculin from focal adhesions. Thus, we concluded that vinculin assembly in focal contacts stabilizes the cell-surface contacts and therefore may be important in the attachment and spreading of cells on artificial substrates *in vitro*. No difference in the assembly of vinculin was found in osteoblasts on the various protein-coated substrates as determined by our immunofluorescence experiments.

From a biomaterial perspective, surface characteristics of implant materials appearing as artificial surfaces may influence the expression of structural proteins. The assembly of vinculin in focal contacts seems to be a prerequisite of osteoblast attachment and spreading on protein-coated and uncoated artificial surfaces. However, it does not seem to have pronounced effects on attachment strength and the subsequent proliferation of cells. To elucidate the mechanisms involved in signal transduction of osteoblast adhesion and growth on artificial surfaces, further cell culture studies are necessary.

References

1. H. P. JENNISSEN, in "Modern aspects of protein adsorption on biomaterials", edited by Y. F. Missirlis and W. Lemm (Kluwer, Amsterdam, 1991) p. 63.
2. R. BENDORI, D. SALOMON and B. GEIGER, *EMBO J.* **6** (1987) 2897.
3. M. J. BISSELL and M. H. BARCELLOS-HOFF, *J. Cell Sci. Suppl.* **8** (1987) 327.

4. L. MASI, A. FRANCHI, M. SANTUCCI, D. DANIELLI, L. ARGANINI, V. GIANNONE, L. FORMIGLI, S. BENVENUTI, A. TANINI, F. BEGHE, M. MIAN and M. L. BRANDI, *Calcif. Tissue Int*. **51** (1992) 202.
5. J. B. MEIGS and Y.-L. WANG, *J. Cell Biol.* **102** (1986) 1430.
6. S. VUKICEVIC, F. P. LUYTEN, H. K. KLEINMAN and A. H. REDDI, *Cell* **63** (1990) 437.
7. W. J. GRZESIK and P. G. ROBEY, *J. Bone Miner. Res.* **9** (1994) 487.
8. K. MOLLER, U. MEYER, D. H. SZULCZEWSKI, H. HEIDE, B. PRIESSNITZ and D. B. JONES, *Cells Materials* **4** (1994) 263.
9. S. J. JONES and A. BOYDE, *Scanning Electron Microsc.* **2** (1979) 529.
10. M. A. MALIK, D. A. PULEO, R. BIZIOS and R. H. DOREMUS, *Biomaterials* **13** (1992) 123.
11. D. A. PULEO and R. BIZIOS, *J. Biomed. Mater. Res.* **26** (1992) 291.
12. *Idem*., *Bone Miner*. **18** (1992) 215.
13. W.-T. CHEN and S. J. SINGER, *J. Cell Biol.* **95** (1982) 205.
14. K. BURRIDGE, K. FATH, T. KELLY, G. NUCKOLLS and C. TURNER, *Ann. Rev. Cell Biol.* **4** (1988) 487.
15. B. GEIGER, Z. AVNUR, G. RINNERTHALER, H. HINSSEN and V. J. SMALL, *J. Cell Biol.* **99** (1984) 835.
16. K. BURRIDGE and J. R. FERAMISCO, *Cell* **19** (1980) 587.
17. J. J. OTTO, *Cell Motil. Cytoskeleton* **16** (1990) 1.
18. M. SAMUELS, R. M. EZZEIL, T. J. CARDOZO, D. R. CRITCHLEY, J.-L. COLL and E. D. ADAMSON, *J. Cell Biol.* **121** (1993) 909.
19. D. B. JONES, H. NOLTE, J. G. SCHOLUEBBERS, E. TURNER and D. VELTEL, *Biomaterials* **12** (1991) 101.
20. S. K. AKIYAMA, K. NAGATA and K. M. YAMADA, *Biochim. Biophys. Acta* **1031** (1990).
21. S. M. ALBELDA and C. A. BUCK, *FASEB J.* **4** (1990) 2868.
22. J. S. BAUER, C. L. SCHREINER, F. G. GIANCOTTI, E. RUOSLAHTI and R. L. JULIANO, *J. Cell Biol.* **116** (1992) 477.
23. R. O. HYNES, *Cell* **48** (1987) 549.
24. E. RUOSLAHTI and M. D. PIERSCHBACHER, *Science* **238** (1987) 491.
25. D. D. SCHLAEPFER, S. K. HANKS, T. HUNTER and P. VAN DER GEER, *Nature* **372** (1994) 786.
26. R. S. CARVALHO, J. E. SCOTT, D. M. SUGA and E. H. YEN, *J. Bone Miner. Res.* **9** (1994) 999.
27. A. MORLA, Z. ZHANG and E. RUOSLAHTI, *Nature* **367** (1994) 193.
28. M. A. HORTON and J. DAVIES, *J. Bone Miner. Res.* **4** (1989) 803.
29. D. A. PULEO and R. BIZIOS, *Bone* **12** (1991) 271.
30. B. GEIGER, *Cell* **18** (1979) 193.

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