ELSEVIER

Biomaterials 22 (2001) 1327–1333

Biomaterials

www.elsevier.com/locate/biomaterials

Enhanced osteoclast-like cell functions on nanophase ceramics

Thomas J. Webster[a,*], Celaletdin Ergun[b], Robert H. Doremus[b], Richard W. Siegel[b], Rena Bizios[a]

[a]*Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA*
[b]*Department of Materials Science and Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA*

Abstract

Synthesis of tartrate-resistant acid phosphatase (TRAP) and formation of resorption pits by osteoclast-like cells, the bone-resorbing cells, on nanophase (that is, material formulations with grain sizes less than 100 nm) alumina and hydroxyapatite (HA) were investigated in the present in vitro study. Compared to conventional (that is, grain sizes larger than 100 nm) ceramics, synthesis of TRAP was significantly greater in osteoclast-like cells cultured on nanophase alumina and on nanophase HA after 10 and 13 days, respectively. In addition, compared to conventional ceramics, formation of resorption pits was significantly greater by osteoclast-like cells cultured on nanophase alumina and on nanophase HA after 7, 10, and 13 days, respectively. The present study, therefore, demonstrated, for the first time, enhanced osteoclast-like cell function on ceramic surfaces with nanometer-size surface topography.

Keywords: Nanophase; Alumina; Hydroxyapatite; Osteoclasts; Orthopaedic/dental

1. Introduction

Healthy bone (which possesses physiological grain sizes of less than 100 nm in diameter [1]) is continuously remodeled through processes that involve formation of a bone modeling unit, activation of bone cells (by the action of growth factors such as insulin-like growth factors I and II [2]), resorption of bone by osteoclasts, and formation of new bone by osteoblasts on the site of the "old" resorbed bone [1]. Osteoclasts, derived from pluripotent (that is, capable of differentiating into various cells including monocytes and macrophages) cells of the bone marrow, resorb bone by forming ruffled cell membrane edges (thereby increasing their surface area of attachment onto bone surfaces), lowering the pH of the local environment by producing hydrogen ions through the carbonic anhydrase system [1] (and, thus, increasing the solubility of hydroxyapatite crystals, the major inorganic component of bone), and lastly, by removing the organic components of the matrix via proteolytic digestion that results in formation of bone resorption pits [1].

Despite the fact that resorption of bone (and, thus, the osteolytic activity of osteoclasts) is an integral part of bone homeostasis, much of the research on biomaterials proposed for orthopaedic/dental implant applications has focused on osteoblast and not osteoclast functions. The extent of bone resorption that occurs at an implant surface determines, at least in part, the fate of a prosthetic device; for example, orthopaedic/dental implant loosening and failure may result either from (i) malnourished juxtaposed bone resulting from little or no resorption of the bone surrounding an implant or from (ii) excessive bone loss (osteolysis) resulting from enhanced resorption of the bone surrounding an implant [3]. Moreover, since osteoblasts are stimulated to deposit calcium-containing mineral by growth factors (such as insulin-like growth factors I and II) secreted by osteoclasts, resorption of bone is soon followed by synthesis of new bone [1]. Undoubtedly, events at the tissue–implant interface that mediate functions of osteoclasts and, thus, the extent of resorption and subsequent formation of new bone around prostheses, determine orthopaedic/dental implant efficacy.

* Corresponding author. Present address: Department of Biomedical Engineering, Purdue University, West Lafayette, IN 47907-1296, USA. Tel.: +1-765-494-1193; fax: +1-765-494-2995.

E-mail address: twebster@ecn.purdue.edu (T.J. Webster).

0142-9612/01/$ - see front matter
PII: S0142-9612(00)00285-4

In vitro functions of osteoclasts have been well documented on conventional orthopaedic/dental materials including ceramics (e.g., hydroxyapatite) [4,5]. For example, compared to devitalized bone, functions (such as decreased synthesis of tartrate-resistant-acid phosphatase (TRAP) and smaller and fewer resorption pits) of osteoclast-like cells decreased on synthetic hydroxyapatite substrates with grain sizes larger than 100 nm [4,5]. To date, functions of osteoclast-like cells on nanophase (that is, material formulations with grain sizes less than 100 nm) ceramics have not been explored. For this reason, the present in vitro study investigated, for the first time, select functions (specifically, synthesis of TRAP and formation of resorption pits) of osteoclasts on nanophase alumina and hydroxyapatite.

2. Materials and methods

2.1. Ceramic substrate synthesis and preparation

Alumina (Al_2O_3) samples (discs 10 mm in diameter and 2 mm thick) were prepared as previously described [6]. Briefly, nanophase alumina (γ-phase; Nanophase Technologies Corporation) powder was compacted in a tool-steel die via a uniaxial pressing cycle (0.2–1 GPa over a 10 min period). Alumina grain size was controlled by changing compact sintering temperatures. Nanophase alumina compacts were sintered at 1000°C for 120 min to obtain final grain sizes less than 100 nm. Alumina compacts were sintered at 1200°C for 120 min to obtain conventional final grain sizes greater than 100 nm.

Hydroxyapatite (HA; $Ca_5(PO_4)_3(OH)$) compacts (10 mm in diameter and 2 mm thick) were prepared via wet chemistry techniques adopted from previously published methods [7]. HA grain size was controlled by changing the time and temperature of HA precipitation; specifically, the HA containing solution was stirred either at room temperature for 24 h to obtain grain sizes less than 100 nm (i.e., nanophase grain size HA) or at 90°C for 3 h to obtain grain sizes greater than 100 nm (i.e., conventional grain size HA). Each HA-containing solution was then centrifuged, filtered, dried at 60°C for 8 h, heated in air at 10°C/min from room temperature to a final temperature of 1100°C, and sintered at this temperature for 60 min.

All ceramic samples were degreased, ultrasonically cleaned and sterilized in a steam autoclave at 120°C for 30 min according to standard laboratory procedures [8].

Devitalized bone slices (1 cm × 1 cm × 1 mm) of bovine femurs were cleaned of adhering tissue and marrow according to established protocols [9] and served as reference substrates. Bone slices were sterilized by soaking in phosphate buffered saline solution supplemented with 1% antibiotic/antimycotic solution (Gibco) at room temperature for 24 h prior to use in experiments with cells; during this time period, the antibiotic/antimycotic containing solution was changed every 8 h.

2.2. Osteoclast-like cell cultures

Rat bone marrow, osteoclast-like cells were obtained using procedures modified from literature reports [10]. Briefly, Wistar rat (2–3 weeks old) femurs were removed, dissected free of tissue, the ends of the tibiae cut off, and the marrow cavities flushed by slowly injecting 3–5 ml of Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic/antimycotic solution at one end of each femur. The isolated marrow tissue was then centrifuged (at $21 \times g$ at room temperature), resuspended in fresh DMEM (containing 10% fetal bovine serum, 10^{-8} M 1-α, 25-(OH_2) vitamin D_3 (Calbiotech) and 1% antibiotic/antimycotic solution) either in the presence or absence of 10^{-8} M calcitonin (Sigma), and seeded under standard cell culture conditions (that is, under a 37°C, humidified, 5% CO_2/95% air environment) on substrates of interest to the present study according to methods described in the sections *Synthesis of tartrate-resistant acid phosphatase, bone resorptive activity*, and *Total intracellular protein synthesis.*

Primary osteoclast-like cells were used in the experiments. These cells were characterized by their multi-nucleated morphology, positive staining for tartrate-resistant acid phosphatase (TRAP), and bone resorptive activity (which was inhibited in the presence of calcitonin).

2.3. Synthesis of tartrate-resistant acid phosphatase

Bone marrow cells (1×10^5 cells) were seeded onto the substrates of interest to the present study and cultured under standard cell culture conditions in DMEM supplemented with 10% fetal bovine serum, 10^{-8} M 1-α, 25-(OH_2) vitamin D_3 (Calbiotech) and 1% antibiotic/antimycotic solution for 7, 10, and 13 days. At the end of the prescribed time periods, adherent cells were rinsed twice with Ca^{2+}- and Mg^{2+}-free phosphate buffered saline, and lysed with distilled water during three freeze–thaw cycles. Tartrate-resistant acid phosphatase (TRAP) activity was then measured in each supernatant containing the lysate of cells according to established protocols [11,12]. Briefly, aliquots from supernatant solutions were incubated with 8 mM *p*-nitrophenylphosphate in 0.1 M sodium acetate, 5 mM ascorbic acid, 0.1% Triton X-100 and 10 mM sodium tartrate (pH = 5.4) (all chemicals from Sigma) at 37°C for 10 min; the reaction of *p*-nitrophenol to *p*-nitrophenylate was stopped by adding 0.05 M NaOH. Light absorbance of these samples was measured on a spectrophotometer (MR600 Spectrophotometric Microplate Reader; Dynatech) at 400 nm. TRAP activity was determined from experimental samples, normalized by total protein synthesis determined as described in

section *Total intracellular protein synthesis*, and expressed as nano-moles of converted *p*-nitrophenol/min/g protein [11,12].

2.4. Bone resorptive activity

Rat bone-marrow cells (1×10^5) were seeded onto the surface of the substrates of interest to the present study and cultured under standard cell culture conditions in DMEM (containing 10% fetal bovine serum, 10^{-8} M 1-α, 25-(OH_2) vitamin D_3 and 1% antibiotic/antimycotic solution) either in the absence or presence of 10^{-8} M calcitonin for 7, 10 and 13 days. At the end of each prescribed time period, the samples were rinsed with ammonium hydroxide, sonicated, and stained with crystal violet. Resorption pits on the ceramic and bone surfaces were visualized using reflected light microscopy (Olympus I × 70; New York/New Jersey Scientific Inc.) with image analysis software (Image Pro); the number of resorption pits formed as a result of the osteolytic activity of osteoclast-like cells were counted [13]. Resorption pit density (number of pits/cm^2) was determined by averaging the number of resorption pits in five random fields (2 mm^2) per substrate. Resorption pits on substrates of interest to the present study were also visualized by scanning electron microscopy (SEM) using a JOEL JSM-840 scanning electron microscope.

2.5. Total intracellular protein synthesis

After 7, 10, and 13 days, osteoclast-like cells cultured on the substrates of interest to the present study (as detailed in *Synthesis of tartrate-resistant acid phosphatase* and *Bone resorptive activity* sections) were rinsed twice with Ca^{2+}- and Mg^{2+}-free phosphate buffered saline and lysed using distilled water and three freeze–thaw cycles. Total protein content in these cell lysates was determined spectrophotometrically using a commercially available kit (Pierce Chemical Co.) and by following manufacturer's instructions. For this purpose, aliquots of each protein-containing, distilled-water supernatant were incubated with a solution of copper sulfate and bicinchoninic acid at 37°C for 30 min. Light absorbance of these samples was measured on a MR600 Spectrophotometric Microplate reader at 570 nm. Protein concentration in each of these samples was determined from standard curves of absorbance versus known concentrations of bovine serum albumin (0–5 mg/ml; Sigma) run in parallel with the experimental samples; the results were expressed as mg/cm^2 of substrate surface area.

2.6. Statistical analysis

Cell experiments were run in triplicate and repeated at three different times per substrate of interest to the present study. Numerical data were analyzed using standard analysis of variance (ANOVA) techniques; statistical significance was considered at $p < 0.01$.

3. Results

3.1. Enhanced synthesis of TRAP by osteoclast-like cells cultured on nanophase ceramics

No TRAP activity was detected when osteoclast-like cells were cultured on either conventional alumina for all time periods tested in the present study (Fig. 1) or on conventional HA for 7 and 10 days (Fig. 2). Compared to respective results obtained after 7 days, synthesis of TRAP by osteoclast-like cells was significantly greater on devitalized bone after 10 and 13 days (Figs. 1 and 2), on nanophase alumina after 10 and 13 days (Fig. 1), on conventional HA after 13 days (Fig. 2), and on nanophase HA after 10 and 13 days of culture (Fig. 2). Compared to respective results obtained after 10 days, TRAP synthesis by osteoclast-like cells was significantly greater on nanophase and conventional HA after 13 days of culture (Fig. 2). Synthesis of TRAP by osteoclast-like cells was similar on either nanophase alumina or devitalized bone, respectively, between 10 and 13 days of culture (Fig. 1).

Synthesis of TRAP was significantly greater when osteoclast-like cells were cultured on devitalized bone (reference substrate) than on either nanophase or

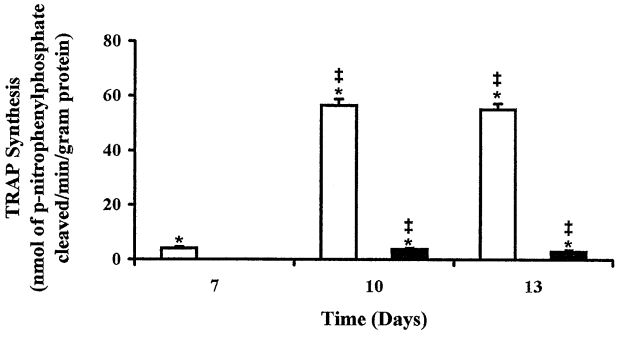

Fig. 1. Synthesis of tartrate-resistant acid phosphatase (TRAP) in osteoclast-like cells cultured on alumina. Synthesis of tartrate-resistant acid phosphatase (TRAP) in osteoclast-like cells cultured in Dulbecco's modified eagle medium (containing 10% fetal bovine serum, 1% antibiotic/antimycotic, and 10^{-8} M 1-α, 25-(OH_2) vitamin D_3) was determined on □ devitalized bovine bone (reference substrate), ▨ 167 nm grain size alumina (conventional), and on ■ 24 nm grain size alumina (nanophase). Compared to results obtained on alumina, synthesis of TRAP was significantly greater when osteoclast-like cells were cultured on devitalized bone for all time periods tested in the present study. More importantly, compared to results obtained on conventional alumina, TRAP synthesis was significantly greater when osteoclast-like cells were cultured on nanophase alumina for 10 and 13 days. TRAP activity on conventional alumina was undetectable. Values are mean ± SEM; $n = 3$; $^*p < 0.01$ (compared to results obtained on 167 nm grain size alumina); $^‡p < 0.01$ (compared to results obtained on respective substrates after 7 days of culture).

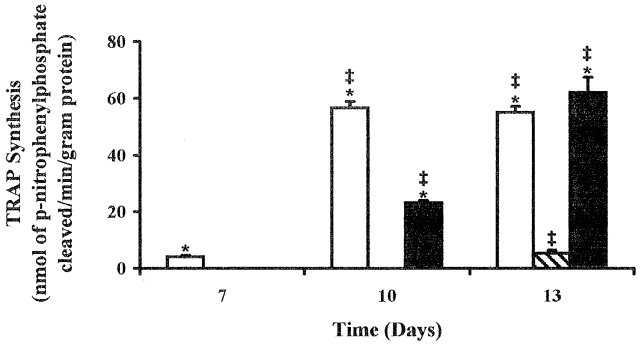

Fig. 2. Synthesis of tartrate-resistant acid phosphatase (TRAP) in osteoclast-like cells cultured on hydroxyapatite. Synthesis of tartrate-resistant acid phosphatase (TRAP) in osteoclast-like cells cultured in Dulbecco's modified eagle medium (containing 10% fetal bovine serum, 1% antibiotic/antimycotic, and 10^{-8} M 1-α, 25-(OH_2) vitamin D_3) was determined on □ devitalized bovine bone (reference substrate), ▨ 179 nm grain size hydroxyapatite (conventional), and on ■ 67 nm grain size hydroxyapatite (nanophase) under standard cell culture conditions (37°C, humidified, 5% CO_2/95% air environment) after 7, 10, and 13 days. Compared to results obtained on nanophase hydroxyapatite, synthesis of TRAP was significantly greater when osteoclast-like cells were cultured on devitalized bone for 7 and 10 days. TRAP synthesis was similar on devitalized bone and on nanophase hydroxyapatite after 13 days. Compared to results obtained on conventional hydroxyapatite, TRAP synthesis was significantly greater when osteoclast-like cells were cultured on nanophase hydroxyapatite for 10 and 13 days. Values are mean ±SEM; $n = 3$; $^{*}p < 0.01$ (compared to results obtained on 179 nm grain size hydroxyapatite); $^{\ddagger}p < 0.01$ (compared to results obtained on respective substrates after 7 days of culture).

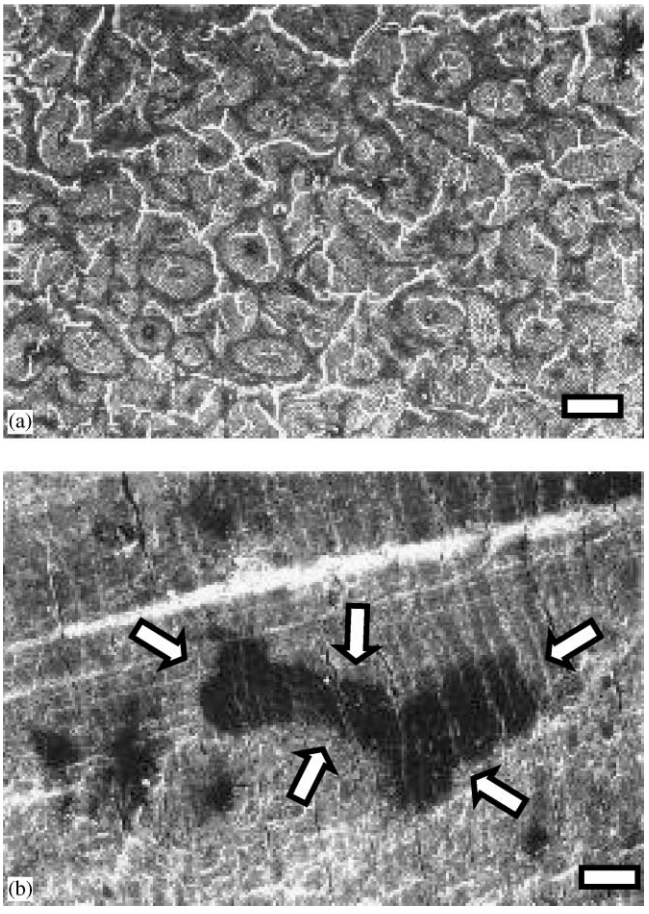

Fig. 3. Representative micrographs of resorption pits formed by osteoclast-like cells cultured on devitalized bovine bone. Scanning electron micrographs illustrating resorption pits formed by osteoclast-like cells cultured on devitalized bovine bone in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum, 1% antibiotic/antimycotic and 10^{-8} M 1-α, 25-(OH_2) vitamin D_3 in either the (a) presence or (b) absence of 10^{-8} M calcitonin under standard cell culture conditions for 13 days. Resorption pits (indicated by arrows on the micrographs) were only formed when osteoclast-like cells were cultured in the absence of calcitonin. *Note: the cracks present on the surface of devitalized bone occurred during sample preparation for scanning electron microscopy after the cell-culture experiments.* Bar = 100 μm.

conventional alumina for all time periods tested in the present study (Fig. 1). Compared to either nanophase or conventional HA, TRAP synthesis was significantly greater in osteoclast-like cells cultured on devitalized bone for 7 and 10 days of culture; after 13 days, however, synthesis of TRAP was similar in osteoclast-like cells cultured on either nanophase HA or on devitalized bone (Fig. 2).

More importantly, compared to respective conventional ceramics, synthesis of TRAP was significantly greater in osteoclast-like cells cultured on nanophase alumina (Fig. 1) and on nanophase HA (Fig. 2) for 10 and 13 days; in fact, compared to results obtained on conventional ceramics after 13 days of culture, TRAP synthesis was five and 12 times greater on nanophase alumina and HA, respectively. Furthermore, compared to results obtained on nanophase alumina, synthesis of TRAP was over 12 times greater when osteoclast-like cells were cultured on nanophase HA for 13 days.

3.2. Enhanced resorption activity by osteoclast-like cells cultured on nanophase ceramics

In the presence of 10^{-8} M calcitonin in the cell culture media, formation of resorption pits (an index of bone resorptive activity) by osteoclast-like cells cultured on devitalized bone slices for 13 days was inhibited (Fig. 3a). Furthermore, no resorption pits were found on any of the substrates of interest to the present study when osteoclast-like cells were cultured in the presence of 10^{-8} M calcitonin in the cell-culture media for either 7, 10 or 13 days (data not shown).

In contrast, in the absence of calcitonin in the cell culture media, resorption pits were formed when osteoclast-like cells were cultured either on devitalized bone (Fig. 3b), alumina (Fig. 4a and b), or on HA (Fig. 4c and d) for 13 days. These resorption pits were larger on nanophase alumina (Fig. 4a) and on nanophase HA (Fig. 4c) compared to those observed on the respective conventional ceramic formulations after 13 days of osteoclast-like cell culture (Fig. 4b and d, respectively).

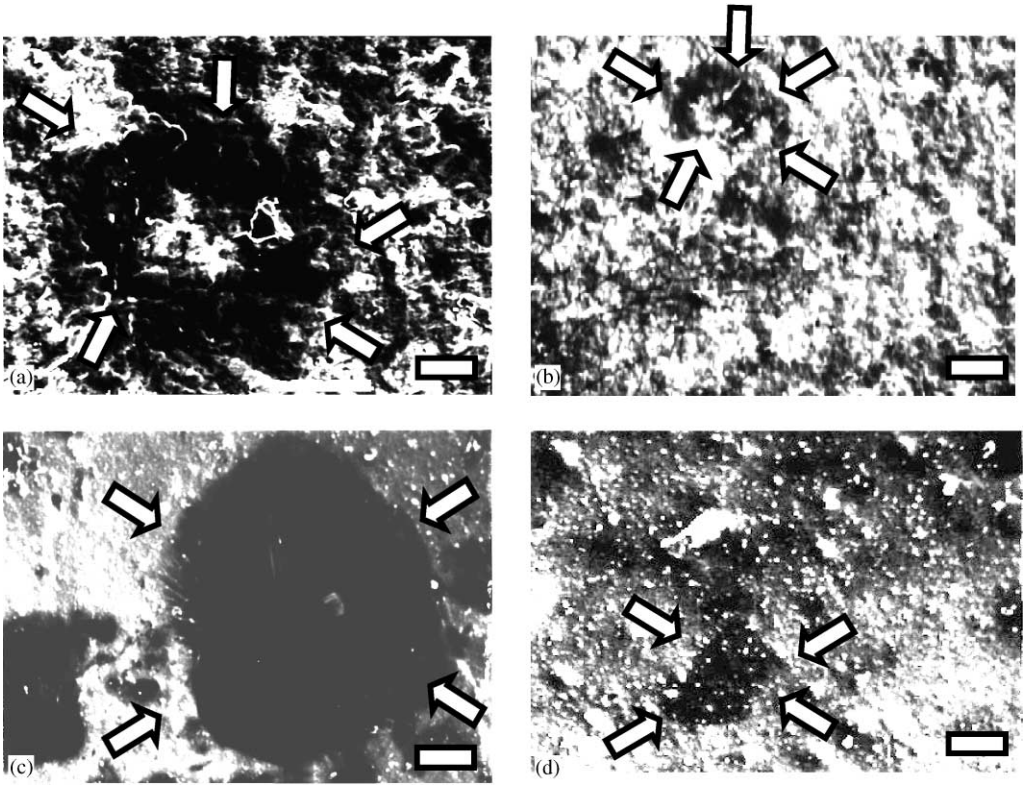

Fig. 4. Representative micrographs of resorption pits formed by osteoclast-like cells cultured on ceramics. Scanning electron micrographs illustrating resorption pits formed by osteoclast-like cells cultured on either (a) nanophase and (b) conventional alumina or on (c) nanophase and (d) conventional hydroxyapatite in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum, 1% antibiotic/antimycotic and 10^{-8} M 1-α, 25-(OH_2) vitamin D_3 under standard cell culture conditions for 13 days. Resorption pits (indicated by arrows on the micrographs) were larger when osteoclast-like cells were cultured on nanophase compared to conventional alumina and hydroxyapatite. Bar = 100 μm.

Compared to respective results obtained after 7 days of culture, the number of resorption pits formed by osteoclast-like cells was significantly greater after 13 days on nanophase and on conventional alumina, respectively (Fig. 5); the number of resorption pits formed by osteoclast-like cells after 7 and 10 days of culture on nanophase alumina were similar. Compared to respective results obtained after 7 days, the number of resorption pits formed was significantly greater when osteoclast-like cells were cultured on nanophase and on conventional HA for 13 days (Fig. 6); the number of resorption pits formed by osteoclast-like cells cultured on conventional HA for 10 and 13 days was similar. There was an increasing (but not statistically different) trend in the number of resorption pits formed by osteoclast-like cells cultured on devitalized bone between 7 and 13 days.

Formation of resorption pits was significantly greater when osteoclast-like cells were cultured on devitalized bovine bone (reference substrate) than either on alumina (Fig. 5) or on HA (Fig. 6) for 7 and 10 days. After 13 days, the number of resorption pits formed by osteoclast-like cells cultured on nanophase alumina (Fig. 5) and on nanophase HA (Fig. 6) was similar to those observed on devitalized bone.

More importantly, compared to conventional ceramics, formation of resorption pits was significantly greater when osteoclast-like cells were cultured on nanophase alumina (Fig. 5) and on nanophase HA (Fig. 6) after all time periods tested in the present study; in fact, compared to results obtained on conventional ceramics after 13 days of culture, the number of resorption pits on nanophase alumina and on nanophase HA were three and four times greater, respectively. In addition, compared to results obtained on nanophase alumina, formation of resorption pits was 25% greater when osteoclast-like cells were cultured on nanophase HA for 13 days.

4. Discussion

In addition to demonstrating, for the first time, enhanced synthesis of tartrate-resistant acid phosphatase (TRAP) and increased formation of resorption pits on nanophase (compared to conventional) alumina and HA, the present study also contributed evidence that may explain these results. Specifically, increased osteoclast-like cell function on nanophase ceramics was

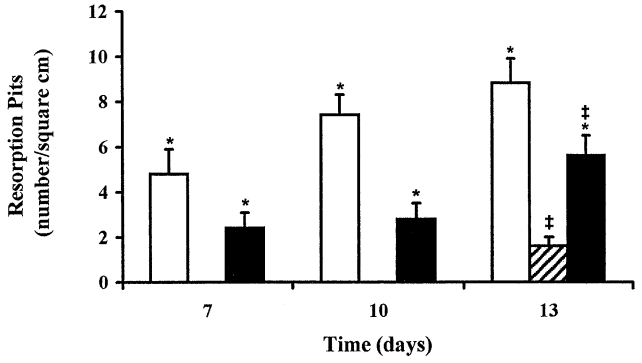

Fig. 5. Time course of resorption activity by osteoclast-like cells cultured on alumina. Resorption pits formed on □ devitalized bovine bone (reference substrate), ▨ 167 nm grain size alumina (conventional), and on ■ 24 nm grain size alumina (nanophase) were quantified following culture of osteoclast-like cells in Dulbecco's modified eagle medium (containing 10% fetal bovine serum, 1% antibiotic/antimycotic, and 10^{-8} M 1-α, 25-(OH_2) vitamin D_3) under standard cell culture conditions (37°C, humidified, 5% CO_2/95% air environment) for 7, 10, and 13 days. Compared to results obtained on alumina, the number of resorption pits was significantly greater when osteoclast-like cells were cultured on devitalized bone after 7 and 10 days; compared to results obtained on nanophase alumina, the number of resorption pits was similar on devitalized bone for 13 days. Compared to results obtained on conventional alumina, the number of resorption pits was significantly greater when osteoclast-like cells were cultured on nanophase alumina for 7, 10, and 13 days. Values are mean ±SEM; $n = 3$; $^{*}p < 0.01$ (compared to results obtained on 167 nm grain size alumina); $^{\ddagger}p < 0.01$ (compared to results obtained on respective substrates after 7 days of culture).

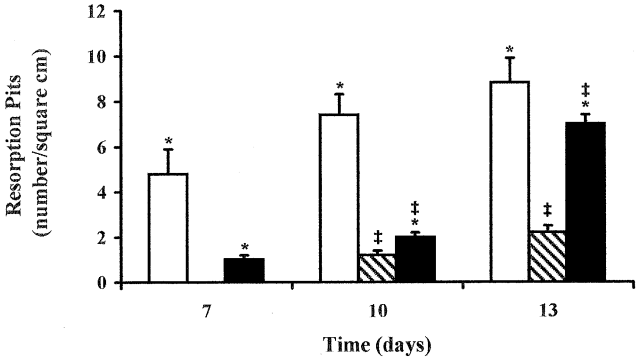

Fig. 6. Time course of resorption activity by osteoclast-like cells cultured on hydroxyapatite. Resorption pits formed on □ devitalized bovine bone (reference substrate), ▧ 179 nm grain size hydroxyapatite (conventional), and on ■ 67 nm grain size hydroxyapatite (nanophase) were quantified following culture of osteoclast-like cells in Dulbecco's modified eagle medium (containing 10% fetal bovine serum, 1% antibiotic/antimycotic, and 10^{-8} M 1-α, 25-(OH_2) vitamin D_3) under standard cell culture conditions (37°C, humidified, 5% CO_2/95% air environment) for 7, 10, and 13 days. Compared to results obtained on nanophase hydroxyapatite, the number of resorption pits was significantly greater when osteoclast-like cells were cultured on devitalized bone for 7 and 10 days. The number of resorption pits was similar on devitalized bone and on nanophase hydroxyapatite after 13 days. Compared to results obtained on conventional hydroxyapatite, the number of resorption pits was significantly greater when osteoclast-like cells were cultured on nanophase hydroxyapatite for 7, 10, and 13 days. Values are mean ±SEM; $n = 3$; $^{*}p < 0.01$ (compared to results obtained on 179 nm grain size hydroxyapatite); $^{\ddagger}p < 0.01$ (compared to results obtained on respective substrates after 7 days of culture).

independent of either ceramic surface chemistry or material phase since enhanced TRAP synthesis and formation of resorption pits was observed on both nanophase alumina and HA. Several explanations for the intriguing phenomenon of enhanced osteoclast-like cell function on nanoceramics may be proposed:

(i) It is plausible that the greater number of resorption pits observed on nanophase ceramics may be due to increased solubility of nanophase compared to conventional alumina and HA by TRAP activity (which is the main acid secreted by osteoclasts actively resorbing the extracellular matrix of bone [14]). Support for this explanation is provided by literature reports of enhanced solubility of chemical species in nanophase compared to conventional materials; for example, the solubility of hydrogen increased by a factor of 10–100 in nano versus larger grain size crystalline palladium [15].

(ii) Enhanced osteoclast-like cell functions on nanophase ceramics may also be due to increased (by 35–50%) surface roughness of nanophase compared to conventional formulations resulting from both decreased surface grain size and decreased diameter of surface pores [16]. Support for this explanation is provided by reports in the literature that demonstrated enhanced synthesis of TRAP and increased formation of resorption pits by osteoclast-like cells cultured on HA substrates of increased micro-size surface roughness [4,5]. The present study, however, was the first to provide evidence of osteoclast-like cell function on surfaces composed of nanophase alumina and HA similar to that observed on devitalized bone.

(iii) In addition to greater surface roughness, nanophase ceramics are characterized by enhanced surface wettability [16] (possibly due to increased surface roughness and/or number of grain boundaries on their surfaces). Increased surface wettability, or hydrophilicity, has been associated with enhanced protein adsorption and, subsequent, cell interaction(s) with biomaterials [17]. For example, previous studies by our research group [18,19] demonstrated that the enhanced wettability of nanophase ceramics corresponded to increased adsorption of vitronectin (a protein which mediates adhesion of osteoblasts [18]) that, subsequently, led to greater osteoblast adhesion on nanophase alumina and hydroxyapatite. The present study provides the first evidence of enhanced osteoclast-like cell function on nanoceramic surfaces of increased wettability.

Surface properties (such as greater roughness [16,18] and increased wettability [16,18]) of nanophase ceramics that mediate enhanced osteoclastic functions constitute parameters that should be taken into consideration when designing and synthesizing the next generation of

orthopaedic/dental implants. Since bone resorption by osteoclasts is accompanied by subsequent deposition of calcium-containing mineral by osteoblasts in vivo [1], the results of the present study imply that enhanced, coordinated functions of osteoclasts and osteoblasts may occur on nanophase ceramics. Such enhanced corresponding events between osteoclasts and osteoblasts may lead to improved osseointegration of orthopaedic/dental implants into juxtaposed bone.

Acknowledgements

The authors wish to thank Ms. Carol Charniga and Dr. Harry Kimmelberg (Division of Neurosurgery Research Laboratory, Albany Medical College) for Wistar rat femurs, the source of the osteoclast-like cells used in the present study, as well as Ms. Michelle Wedig (Department of Biomedical Engineering, Rensselaer Polytechnic Institute) for assistance with scanning electron microscopy. The contribution of nanophase alumina powder by Nanophase Technologies Corporation is also acknowledged.

References

[1] Kaplan FS, Hayes WC, Keaveny TM, Boskey A, Einhorn TA, Iannotti JP. In: Simon SP, editor. Orthopaedic basic science. Columbus, Ohio: American Academy of Orthopaedic Surgeons, 1994. p. 127–85.

[2] Heegard A. Structure and molecular regulation of bone matrix proteins. J Bone Miner Res 1993;8:S843–7.

[3] Brunski JB. In: Davies JE, editor. The bone-biomaterial interface. Toronto: University of Toronto Press, 1991. p. 391–404.

[4] Gomi K, Lowenberg B, Shapiro G, Davies JE. Resorption of sintered synthetic hydroxyapatite by osteoclasts in vitro. Biomaterials 1993;14(2):91–6.

[5] Matsunaga T, Inoue H, Kojo T, Hatano K, Tsujisawa T, Uchiyama C, Uchida Y. Disaggregated osteoclasts increase in resorption activity in response to roughness of bone surface. J Biomed Mater Res 1999;48(4):417–23.

[6] Webster TJ, Siegel RW, Bizios R. Design and evaluation of nanophase alumina for orthopaedic/dental application. Nanostruct Mater 1999;12:983–6.

[7] Jarcho M, Bolen CH, Thomas MB, Bobick J, Kay JF, Doremus RH. Hydroxylapatite synthesis and characterization in dense polycrystalline form (for bone and tooth implants). J Mater Sci 1976;11(11):2027–35.

[8] Puleo DA, Holleran LH, Doremus RH, Bizios R. Osteoblast responses to orthopaedic/dental materials in vitro. J Biomed Mater Res 1991;25:711–23.

[9] Burkstrand M. Bone cell adhesion on substrates modified with immobilized bioactive peptides. Master's thesis, Rensselaer Polytechnic Institute, Troy, NY, 1996.

[10] Takahashi K, Mishima K, Ichikawa Y, Watanabe K, Komatsuda M, Arimori S. Report of a case with chronic myelomonocytic leukemia: demonstration of leukemic monocytes lacking nonspecific esterase by flow cytometry using monoclonal antibodies. Tokai J Exp Clin Med 1987;12(5–6):275–81.

[11] Lieberherr M, Vreven J, Vaes G. The acid and alkaline phosphatases, and phosphoprotein assay. Biochem Biophys Acta 1973;293:160–9.

[12] Lindunger A, MacKay CA, Ek-Rylander B, Andersson G, Marks Jr SC. Histochemistry and biochemistry of tartrate-resistant acid phosphatase (TRAP) and tartrate-resistant acid adenosine triphosphatase (TrATPase) in bone, bone marrow and spleen: implications for osteoclast ontogeny. Bone Miner 1990; 10(2):109–19.

[13] Nichols KG, Puleo DA. Effect of metal ions on the formation and function of osteoclastic cells in vitro. J Biomed Mater Res 1997;35(2):265–71.

[14] Rifkin BR, Gay CV. Biology and physiology of the osteoclast. Boca Raton, FL: Academic Press, 1992.

[15] Mutschele T, Kirchheim R. Segregation and diffusion of hydrogen in grain boundaries of palladium. Scripta Metallurgica 1987; 21(2):135–40.

[16] Webster TJ, Siegel RW, Bizios R. Osteoblast adhesion on nanophase ceramics. Biomaterials 1999;20:1221–7.

[17] Horbett TA. Principles underlying the role of adsorbed plasma proteins in blood interactions with foreign materials. Cardiovascular Pathol 1993;2:137S–48S.

[18] Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics. J Biomed Mater Res 2000;51:475–83.

[19] Webster TJ, Schadler LS, Siegel RW, Bizios R. Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin. Tissue Engng 2001, in press.