Metabolic heat production as a measure of macrophage response to particles from orthopedic implant materials

S. J. Charlebois, A. U. Daniels, R. A. Smith
University of Tennessee—Campbell Clinic, Department of Orthopaedic Surgery, 956 Court Avenue, Room A302, Memphis, Tennessee 38163

Received 2 March 2001; revised 23 May 2001; accepted 4 June 2001

Abstract: An *in vitro* method to gauge metabolic heat response of macrophages (MØ) to particulates is described. Whereas the majority of work cited relies on chemical analysis to assess MØ response to particles, we have used isothermal microcalorimetry (IMC) for direct continuous measurement of metabolic heat production to gauge the response. IMC is a screening method, in that it ensures that no energy-consuming phagocytic response goes undetected, and that the aggregate metabolic magnitude of the responses is determined. A four-well IMC was used in all microcalorimetric measurements. To accommodate "zero-time" monitoring of the interaction of particles and cells, a set of identical test chambers was constructed for use in the IMC. MØs were injected from outside the IMC onto particles contained in collagen or gelatin on glass coverslips at the bottom of each chamber. IMC runs were performed using MØs only, MØs and lipopolysaccharide (LPS) positive control, and MØs and clean or LPS-bound particles of either high-density polyethylene (HDPE) or cobalt–chrome alloy (CoCr). Total heat produced by the negative controls (MØs alone) was lower than for MØ exposure to LPS or particles. The trend was a higher response for LPS-bound HDPE compared with clean HDPE particles, though not significant. In conclusion, our results have shown that IMC can be used to detect the heat associated with the phagocytosis of particulate materials by MØs *in vitro*. © 2001 John Wiley & Sons, Inc. J Biomed Mater Res 59: 166–175, 2002

Key words: isothermal microcalorimetry; osteolysis; macrophage; particulates; UHMWPE; cobalt chrome alloy; titanium; bone; inflammatory response; cytokines

INTRODUCTION

Aseptic loosening in total arthroplasty is a serious problem, accounting for approximately 75% of revision procedures.[1,2] Much research has focused on understanding the underlying causes of osteolytic bone loss. One mechanism for osteolysis (Fig. 1) appears to involve an inflammatory response to micron and submicron particulate debris shed from the metal and polymer surfaces of the total joint implant.[3–12]

Results from Swedish work[13,14] with dialysis membranes, zymosan particles and granulocytes, and the cited studies stimulated us to think that IMC might be adapted to study the metabolic response of macrophages (MØs) to orthopedic implant wear particles. If an isothermal microcalorimetry (IMC) technique could be developed, it might serve as an *in vitro* screening tool for studying the response of MØs to various particle variables (e.g., type, size, morphology) and for related studies of biologic variables. MØ responses can, of course, be evaluated periodically from amounts of cytokines synthesized (PCR) and/or secreted (ELISA), but assay choices must be made. For screening, we believe that continuously measuring the overall metabolic response has merit.

The main objective of this study was to develop and demonstrate a new method for studying cellular interactions with particulate debris derived from orthopedic implant materials. In addition to the proof of concept aspect of the work, we also intended to determine the relative MØ inflammatory response to two types of particles, with and without bound lipopolysaccharide (LPS) bacterial debris. Recent reports from the literature and work underway in our laboratories suggest that the presence of LPS adsorbed to the surface of particles may exacerbate the inflammatory response of macrophages and other phagocytic cells.[15,16]

Correspondence to: S. J. Charlebois; e-mail: steven.charlebois@Zimmer.com

Contract grant sponsor: NIH NIAMSD; contract grant number: 1R41-AR44581-01

MATERIALS AND METHODS

Cells and cell culture conditions

A transformed mouse peritoneal MØ cell line (IC-21, TIB-186; ATCC, Rockville, MD) was selected because it is well

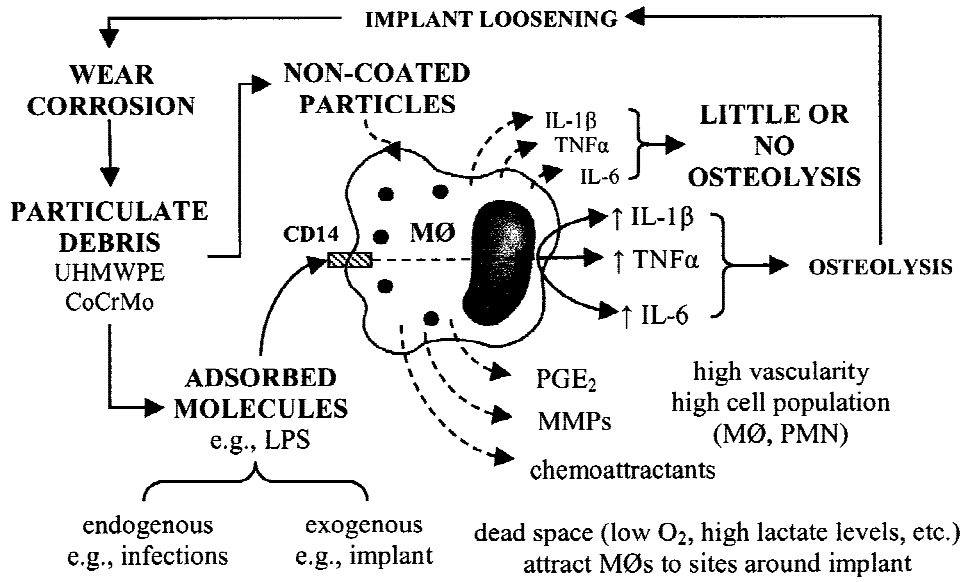

Figure 1. Schematic diagram depicting the role of the MØ in osteolysis, the cytokine signaling involved, and the positive feedback cycle that ultimately results in aseptic loosening of the total joint components.

characterized and readily available. These phagocytic MØs are capable of secreting cytokines and lysosomal enzymes, and do not require the special isolation and purification procedures required of primary cultures.[17]

An important consideration in choosing peritoneal MØs for the IMC experiments was the ability of these cells to function in a low O_2 environment.[18,19] Also, the IC-21 MØ cell line has been used by others[8,20] to study the cellular response to particles relating to osteolysis and aseptic loosening of total joint components.

MØs were maintained in polystyrene tissue culture flasks (CoStar, Cambridge, MA) in RPMI-1640 medium (Gibco BRL, Grand Island, NY) supplemented with 5% fetal bovine serum (Sigma, St. Louis, MO), 1% gentamicin (Gibco BRL), and 2 m*M* L-glutamine (Sigma), at 37°C in a 5% CO_2 atmosphere with 100% humidity.

Immediately before each IMC experiment, MØs were collected by incubation with calcium and magnesium-free phosphate buffered saline, centrifuged for 5 min at 190*g*, and resuspended in fully supplemented medium. MØ cell count estimates were performed with a SPotlite™ hemacytometer (American Scientific Products, McGaw Park, IL).

Implant material particles

Micron-sized particles of two different orthopedic implant materials—high-density polyethylene (HDPE) and cobalt–chrome alloy (CoCr)—were used in these studies (Smith & Nephew Orthopaedics, Memphis, TN). We used HDPE rather than ultrahigh molecular weight polyethylene (UHMWPE) particles because well-characterized, micron-range-sized HDPE particles were readily available. Although UHMWPE is more commonly used, HDPE is also used clinically in joint replacements and, indeed, some of the best long-term clinical wear resistance results reported[21] have been for radiation crosslinked HDPE. It is possible that HDPE and UHMWPE may differ in the nature of their adsorption of biologic substances (e.g., endotoxin) to the surface. It does not seem likely that the difference will be gross (except possibly for formulations with additives such as stearates). However, surface atomic structure and resultant adsorption affinities may be somewhat different because UHMWPE has inherently lower crystallinity and density than HDPE. The CoCr particles used in this study were produced in total joint wear simulators.

The sources and size properties of the particles used are provided in Table I. As shown, the particles were a few microns in diameter and similar in mean size and distribution. Particles in this range may be taken up by MØs, and are reported as typical for clinically retrieved total hip wear debris.[22–24]

Particle-coated glass coverslip preparation

Coverslips (Fisher Scientific, Pittsburgh, PA) were coated with either 200 μL of 0.4% type I collagen (BI, from bovine skin; a gift from the Department of Rheumatology, University of Tennessee, Memphis, TN) or 50 μL of 10% gelatin (GEL, type B from bovine skin; Sigma). BI or GEL entrapment of particles is necessary with HDPE to prevent them from floating, and also serves to evenly disperse the relatively heavy metal particles across the coverslip.

At the outset of this work, MØ-particle experiments were performed using the 0.4% BI substrate, as per the method described in a recent publication by Voronov et al.[8] As part of our IMC method development, and because BI readily degrades from its native state especially at 37°C, 10% gelatin was tested as an alternative for suspending particles for MØ phagocytosis.

HDPE and CoCr particles, with and without adsorbed LPS, were suspended in the BI and GEL solutions such that, in the subsequent experiments, the coated volume resulted

TABLE I
Summary Information for HDPE and CoCr Particles Used in IMC Experiments

Particle Type	Industry Source	Density* (g/cm^3)	Measured Diameter, Mean ± SD (μm)	Estimated[a] No. of Particles per Gram	Estimated[b] Surface Area per Gram
High-density polyethylene (HDPE)	Shamrock Technologies, Newark, NJ	0.955	4.73 ± 2.11	1.89×10^{10}	$13.3 + 10^9$
Cobalt-chrome alloy (ASTM F-75) (CoCr)	Smith & Nephew Orthopaedics, Memphis, TN	7.70	2.49 ± 1.45	1.62×10^{10}	3.2×10^9

[a]Density values for materials referenced from the literature.
[b]Computations to estimate number of particles and surface area per gram assumed that particles were spheres, and were made using the above literature-referenced densities and measured mean particle diameters (reported by the sources providing the particles).

in a cell-to-particle ratio of approximately 1:1. LPS binding to cleaned HDPE or CoCr particles was performed in filter units (Ultrafree-MC Centrifugal Filters with a 0.22-μm Durapore® membrane; Millipore Corp., Bedford, MA), and is described in detail elsewhere.[25]

Coverslips coated with BI or GEL alone were used as negative controls in the IMC experiments. LPS from *Escherichia coli* (Sigma) was added at a concentration of 0.78 μg/mL directly to the cells, and was used in positive control experiments.

All coverslips were prepared the night before IMC experiments and allowed to dry under a laminar flow hood with the ultraviolet light on to sterilize the coated coverslips. To begin an experiment, a coated coverslip was placed in the bottom of each experimental chamber, and 1.5 mL of RPMI medium was added. The chambers (described below) were then inserted into the IMC.

Microcalorimeter/chamber design

A four-well isothermal microcalorimeter (model 4400 IMC; Calorimetry Sciences Corp., Provo, UT) was used throughout the IMC experiments, which were performed at 37°C.

Special IMC test chambers were developed for the MØ-particle experiments in collaboration with Calorimetry Sciences Corporation. To minimize heat effects due to extraneous degradation or corrosion reactions, the cylindrical IMC chambers (75-mm height × 30-mm diameter) were constructed from Hastalloy™ metal alloy. Each chamber had a Hastalloy screw-on cover with an attached stainless steel external access tube. Thermal shunts were mounted along the length of the access tube. The access tube was fitted internally with 18-gauge polyethylene tubing, which was attached to a 5-mL syringe. This design allowed eventual injection of MØs in a small volume of medium via the syringe–PE tubing combination, after the metal chamber containing the coverslip and entrapped particles was thermally stabilized in the IMC.

IMC experimental method

In preparation for IMC experiments, the inside of each chamber was wiped clean with 90% ethyl alcohol, and then rinsed several times with phosphate buffered saline. Clean chambers were wrapped in aluminum foil and autoclave sterilized. Final assembly of IMC chambers (including insertion of the particle-coated coverslip) was performed in a laminar flow hood.

Thermal stabilization of the assembled chambers was performed in two steps. First, chambers were placed in an incubator oven at 37°C for approximately 30 min. Then, chambers were quickly transferred to the IMC where further stabilization was achieved by suspending the chambers just above the measurement level of the IMC well. After dropping chambers into the measurement position of the well, another 5 to 10 h was required before steady-state heat flow rate data were obtained.

After sterilization, sterile syringes attached to 18-gauge sterile tubing were used to deliver approximately 1.25 million MØs suspended in 1 mL of 5% RPMI medium into each IMC chamber. Heat flow rate data were then taken over the next 25 h. At the end of each IMC experiment, supernatants were retained for cytokine analysis and glass coverslips containing cells and particles were examined for changes in morphology and extent of phagocytosis. Commercially available ELISA kits (R&D Systems, Minneapolis, MN) were used to analyze supernatants for interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α released by the mouse MØs.

Statistical analysis

The two-tailed student *t* test was used to determine the effect of MØ concentration, adding LPS (positive control), and incubating with clean and LPS-bound particles on metabolic heat production.

EXPERIMENTAL RESULTS

Cell concentration experiments

For the cell concentrations examined (100 k, 500 k, and 1000 k per mL), and for each adhesion substrate

(BI and GEL), the relationship with heat flow rate was linear to a first approximation. This linear relationship was maintained throughout the duration of the IMC experiment (24 h). Figure 2 summarizes the MØ heat flow rate data from repeated BI and GEL experiments at the three cell concentrations. The cell concentration experiments were all performed with MØs harvested from flasks that had been initially subcultured from the same flask of confluent MØs.

Based on these initial results, it was decided that 1.25 million cells in 2.5 mL (500 k per mL) of culture media would be an appropriate concentration for subsequent particle experiments.

Negative and positive control experiments

Figure 3 shows typical IMC heat flow rate recordings from blank injection (RPMI medium), negative control (RPMI + MØs), and positive control experiments (RPMI + MØs + LPS). Thermal stability of the IMC chambers was achieved approximately 3 to 5 h after delivering 1 mL of suspended MØs. The blank injection experiment results demonstrate that, although a slight 5 to 10 μW transient heat effect does occur over the first 3 to 5 h because of opening the chambers to deliver cells, subsequent heat flow data return to the baseline.

In the MØ-only negative controls, the heat flow rates increased over the first 3 to 5 h (after MØs were added), and then gradually decreased with time through the end of the experiment. A summary bar graph (see Fig. 4) shows heat flow rate data from repeated BI and GEL experiments, using MØs from flasks that had been initially subcultured from the same confluent flask. The mean heat flow rates at each time point are similar for the two adhesion substrates, with the standard deviations slightly higher for the BI-coated coverslips. At 20 h after MØs were added to

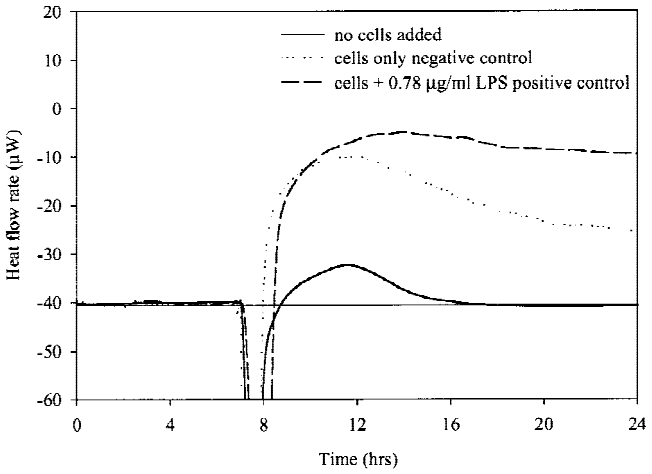

Figure 3. Representative heat flow recordings for MØs on BI: (solid line) simulation experiment (no MØs added), (dotted line) "cells only" negative control, and (dashed line) with 0.78 μg/mL LPS positive control.

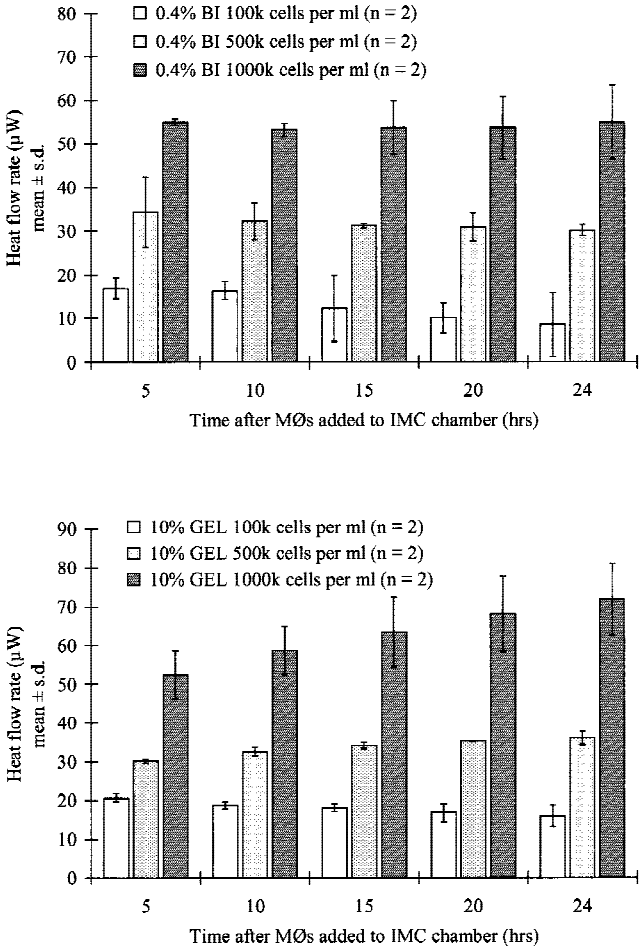

Figure 2. Summary of heat flow rate data for IMC experiments: MØs-only negative controls at different concentrations on BI- and GEL-coated coverslips.

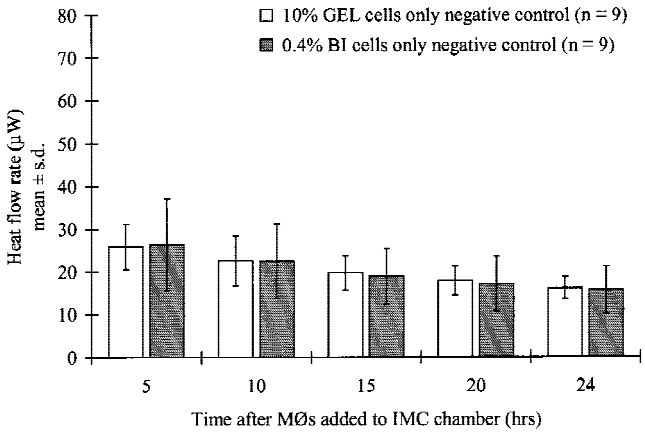

Figure 4. Summary of heat flow rate data for IMC experiments: MØs-only negative controls on BI- and GEL-coated coverslips.

the IMC chambers, the heat flow rate measured for cells on GEL was 17.9 ± 3.3 μW, whereas the corresponding value for cells on BI was 17.2 ± 6.4 μW. In subsequent IMC experiments, to account for the variability in actual numbers of MØs present and overall condition of cells harvested from culture, a cells-only negative control was included as one of the three tests performed during each IMC experiment.

The metabolic response of MØs to the LPS positive control remained relatively constant with time for both substrates (see Fig. 5). At 20 h, the heat flow rate measured for the GEL positive control was 42.8 ± 5.9 μW (approximately 13 μW more exothermic than the respective 20-h negative control value), whereas the corresponding BI positive control value was 38.4 ± 12.5 μW (approximately 20 μW more exothermic than the 20-h negative control value). Statistically significant differences ($p < 0.05$) were obtained between the negative and LPS positive control heat flow values at each time point for the GEL-coated slips. The difference in the positive control data for BI-coated slips was less apparent ($p < 0.15$) because of a larger standard deviation for each time point.

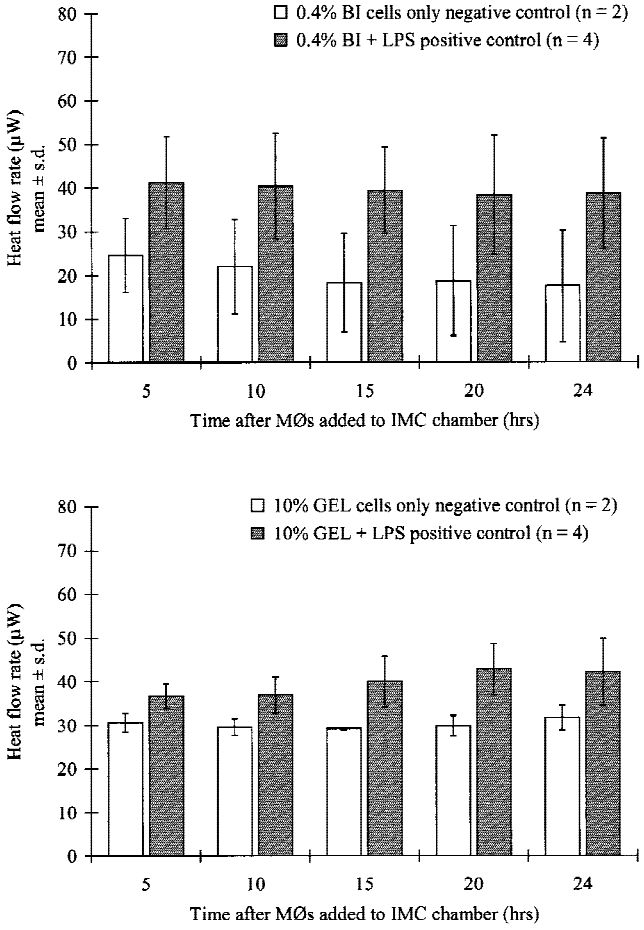

Figure 5. Summary of heat flow rate data for IMC experiments: MØs + 0.78 μg/mL LPS in solution on BI- and GEL-coated coverslips.

CoCr particle experiments

Heat flow rate recordings obtained for MØs + cleaned and LPS-bound CoCr alloy particles in BI were found to reach a virtual steady state after a few hours, as demonstrated in the representative curves (for GEL substrate only) shown in Figure 6. The corresponding IMC experiments performed with GEL resulted in curves that exhibited a slight decrease in heat flow rate over time for both types of particles.

The results for BI and GEL CoCr particle experiments are summarized in Figure 7. The presence of CoCr particles in BI or GEL increased the heat flow rate with respect to the baseline negative control value by 15 to 20 μW, a difference that was significant at each time point ($p < 0.05$). At 20 h, the heat flow rate measured for MØs + clean and LPS-bound CoCr in GEL was 24.7 ± 4.3 and 28.1 ± 5.7 μW, respectively; for clean and LPS-bound CoCr in BI, the heat flow rate was 47.7 ± 8.3 and 46.0 ± 9.7 μW, respectively. There was no statistical difference in MØ response to the ethanol-cleaned versus LPS-bound CoCr particles for either substrate.

HDPE particle experiments

Typical heat flow recordings for the HDPE particle experiments (shown for GEL substrate only) are shown in Figure 8. As in the case of the CoCr curves, the HDPE recordings exhibit a virtually steady-state heat response after the first few hours.

Once again, the difference between the HDPE particle-exposed cells and the cell-only negative controls proved to be statistically significant ($p < 0.05$). The

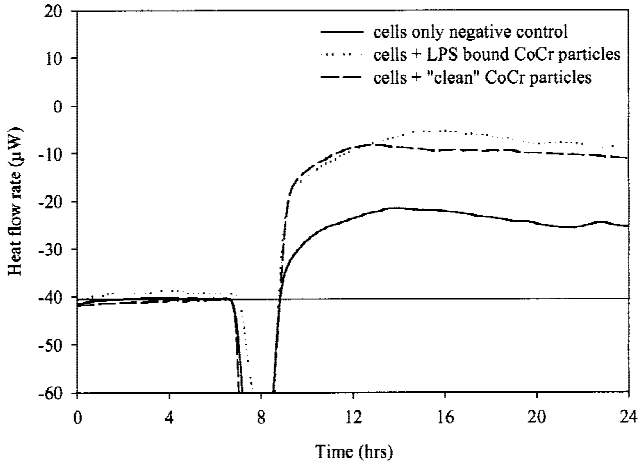

Figure 6. Representative heat flow recordings for MØs on GEL: (solid line) "cells only" negative control, and with suspended (dashed line) clean CoCr, and (dotted line) LPS-bound CoCr particles.

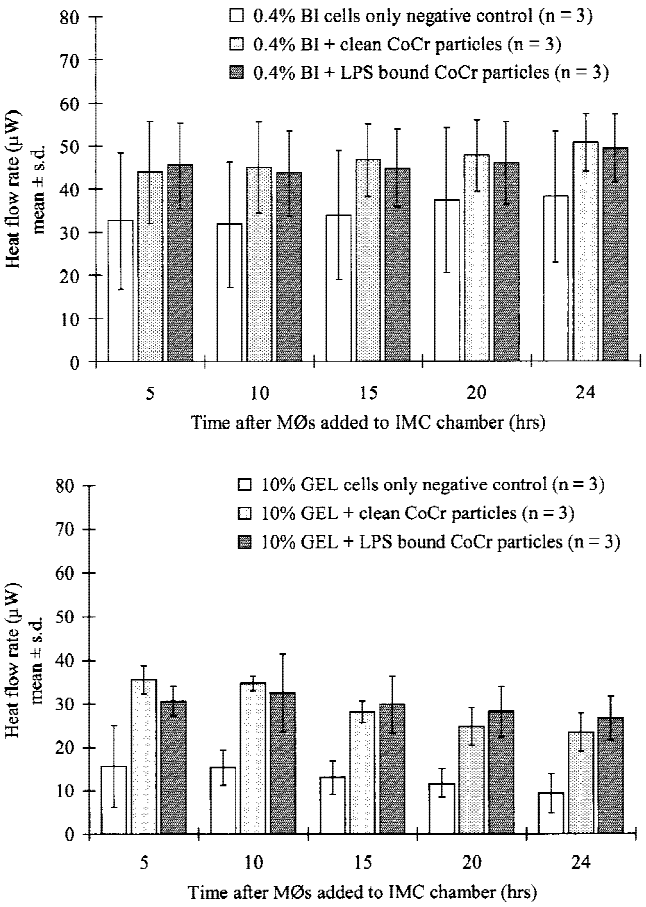

Figure 7. Summary of heat flow rate data for IMC experiments: MØs + clean and LPS-bound CoCr particles on BI- and GEL-coated coverslips.

difference between MØ response to clean and LPS-bound HDPE particles, although not significant, does suggest a trend ($p < 0.15$) toward an increased response for LPS-bound particles at each time point (see Fig. 9). At 20 h, the heat flow rate measured for MØs exposed to clean and LPS-bound HDPE in GEL was 39.6 ± 15.7 μW and 43.0 ± 14.2 μW, respectively; for clean and LPS-bound HDPE in BI, the heat flow rate was 41.5 ± 12.1 μW and 46.2 ± 17.3 μW, respectively.

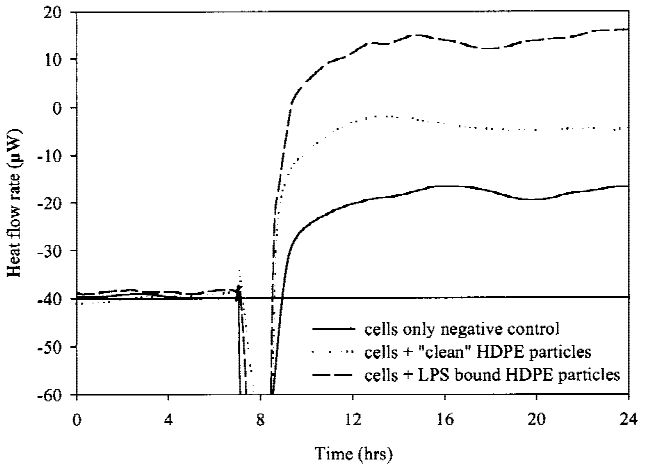

Figure 8. Representative heat flow recordings for MØs on GEL: (solid line) "cells only" negative control, and suspended (dotted line) clean HDPE, and (dashed line) LPS-bound HDPE particles.

Cytokine analysis

Cytokine secretion data are summarized in Figure 10. The levels of TNF-α and IL-6 measured in medium for the LPS (positive control) and the implant particle exposures were significantly higher ($p < 0.05$) compared with the MØ-only negative control after 24 h. Differences between clean and LPS-bound particles of CoCr suspended in BI and GEL were also significant for TNF-α and IL-6. For HDPE particles, only TNF-α for the BI-coated coverslips was significantly different. IL-1β levels were not significantly different between any of the particle-exposed cells and the controls.

DISCUSSION

The purpose of this study was to develop and assess IMC as a new method to study macrophage response

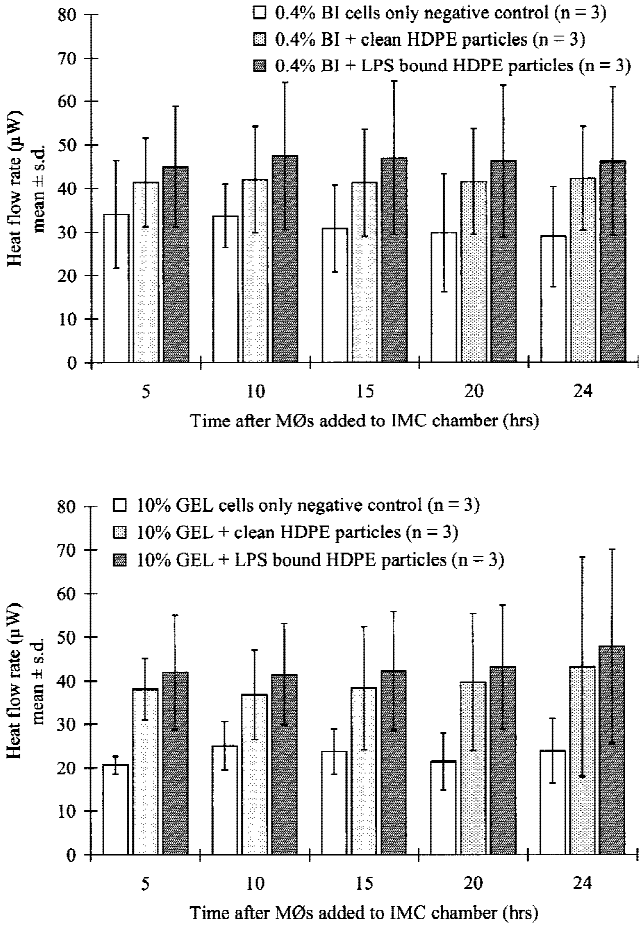

Figure 9. Summary of heat flow rate data for IMC experiments: MØs + clean and LPS-bound HDPE particles on BI- and GEL-coated coverslips.

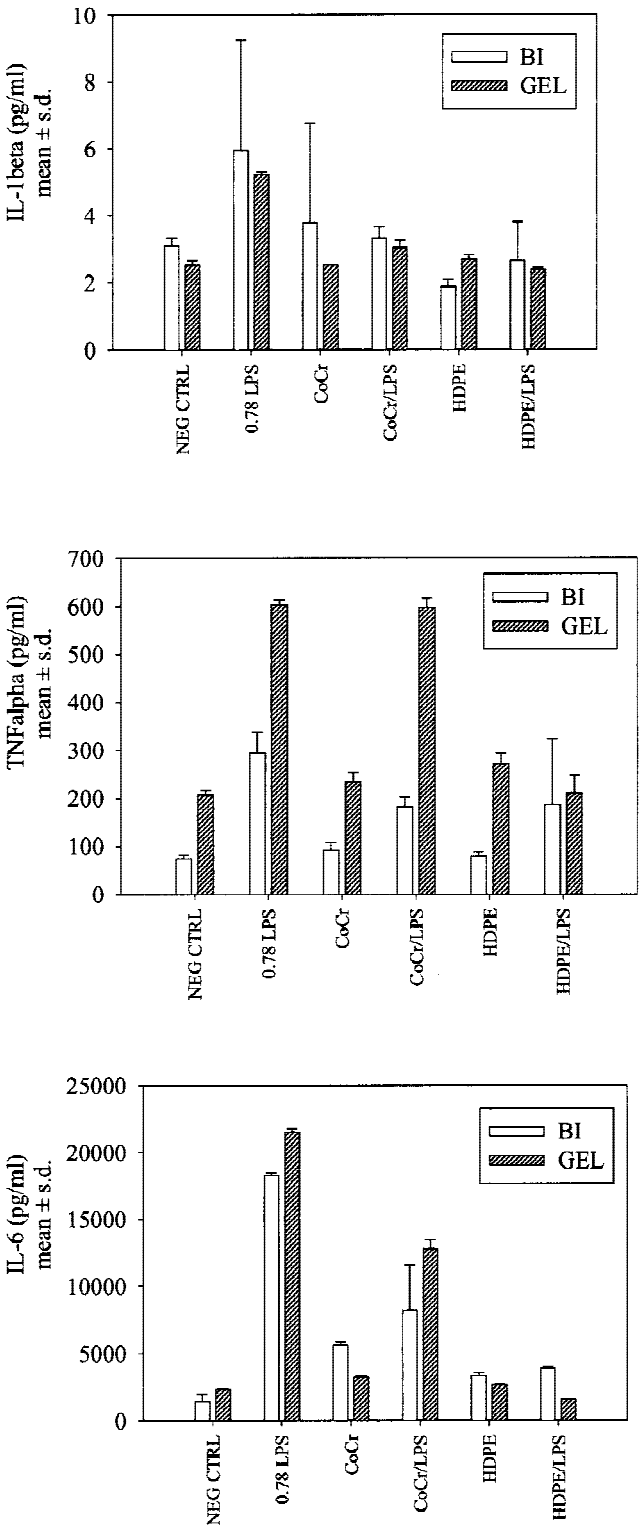

Figure 10. Summary of macrophage TNFα, IL-6, and IL-1β secretion in response to LPS and particulate stimuli for both BI and GEL substrates.

to particulate debris from orthopedic implant materials *in vitro*. Because metabolic heat is produced in all cellular biochemical processes, IMC measurement of exothermic heat flow rate is a general technique for studying the overall magnitude of metabolic response.[26–34] A general method, such as IMC, is also particularly well suited to the detection of unknown cell metabolic phenomena.[35–42] In the present study, significant heat flow rate differences were demonstrated for MØs cultured with endotoxin or particulates compared with cells cultured without artificial stimuli.

At the cell concentrations investigated, and for the amount of medium and headspace maintained in the IMC test chamber, the amount of oxygen available to the cells would not have been appreciably depleted, and the MØs used can function in a low oxygen environment. Heat flow rate values for MØs cultured in monolayers on BI- or GEL-coated coverslips was shown to depend on cell concentration. A linear increase in heat production with cell concentration was observed for both adhesion substrates over the concentration range studied.

The magnitude of heat output of cells is highly dependent on the initial and relative flux of glucose via metabolic pathways (i.e., anaerobic and hexose monophosphate shunt).[14] Assuming that most of the oxygen present is used to oxidize glucose to form $CO_2 + H_2O$ (in the MØs), an estimate of the rate of metabolic heat production can be made. Thoren et al.[32] used the previously determined value for the mean basal respiratory rate of rabbit alveolar MØs and known enthalpy for the oxidation of glucose to estimate total heat output per MØ (18.1 pW). The results of Thoren et al. further proved that alveolar MØs become more peritoneal-like when cultured under O_2-limited conditions, and especially when supplemented with serum. Table II shows a summary of the results from selected studies from the literature in which microcalorimetry was used to determine cellular heat production. Our mean heat production measured for MØs cultured on BI- and GEL-coated coverslips 20 h after cells were added to the IMC chamber was 20.6 ± 10.1 and 16.2 ± 3.1 pW/cell, respectively. These values compare well with the previously cited heat flux values reported by Thoren et al.[32] for monolayer cultured alveolar MØs (18.1 pW/cell).

A gradual decline in the heat output of cells cultured on both substrates without endotoxin or particles was observed. There was no statistical difference between heat output for cells cultured on BI versus GEL. However, variability in heat flow rate values for MØs cultured on the BI-coated coverslips was higher. This may be due to a response to the degradation products of BI and/or to degradation of the BI substrate itself.

The typical response for MØs exposed to 0.78 μg/mL LPS alone (no particles) was a rapid initial increase in heat output, followed by steady-state heat production through the end of the IMC experiment. This behavior is presumably because LPS is mixed with the MØs before injection into the IMC, and thus is imme-

TABLE II
Heat Production Rate (pW per Cell) Measured for Different Cell Types Referenced from the Literature

Reference	Calorimetric Technique	Cell Line	Suspended or Monolayer	Heat Rate, Mean ± SD (pW/cell)
Hoffner et al., 1985[33]	Flow microcalorimeter	L929 mouse fibroblast	Suspended	34 ± 3
Valdermarrson et al., 1990[29]	IMC	Human lymphocytes	Suspended	2.31 ± 0.12
Nassberger et al., 1986[31]	IMC	Rat hepatocytes	Monolayers	327 ± 13
Ikomi-Kumm et al., 1991[14]	IMC	Human granulocytes	Suspended	1.47 ± 0.31
Thoren et al., 1990[32]	IMC	Rabbit alveolar macrophages	Monolayers with serum	27.0 ± 2.0
			Monolayers without serum	19.0 ± 3.2

diately accessible to the cells. In contrast, the MØs must "seek out" particles embedded in the BI or GEL substrates over time.

The heat response of MØs to particles with LPS bound to the surface versus ethanol-cleaned particles was not statistically significant in the data presented herein. In addition, irrespective of whether the particles were cleaned or bound with endotoxin, the metabolic heat response of MØs to GEL-suspended particles was generally slightly lower than the response to particles in BI. This may be explained by subtle differences in each substrate's ability to suspend particles. In GEL, most of the CoCr particles sank through the GEL to rest on the coverslip. Although no significant difference due to LPS binding exists in the CoCr particle treatments, there appears to be a trend in the HDPE particle data that suggests differences between MØ response to clean and LPS-bound particles. Also, HDPE particles did appear to be less clumped and better distributed throughout the GEL than in the BI coatings, and this may have increased chances for exposure of MØs to bound LPS.

Evidence of particle phagocytosis is demonstrated by the light and confocal microscope images shown (for CoCr particles) in Figure 11. Light microscopy showed that the MØs were highly associated with particulate debris after the 24-h IMC runs. The confocal images (z-series) were obtained to demonstrate that, in fact, particles were internalized by the cells and not merely attached to the surface. No significant difference in cell morphology or proliferation was observed for MØs exposed to clean or LPS-bound particles.

One of the major drawbacks of the present work is the limited number of replicate runs. This is a consequence of the small number of test wells in current IMC designs. This limitation on the speed with which replicate runs for a given set of variables can be made is particularly important given the inherent variability of cells in culture

CONCLUSION

Isothermal microcalorimetry is sufficiently sensitive to detect the heat of phagocytosis associated with the

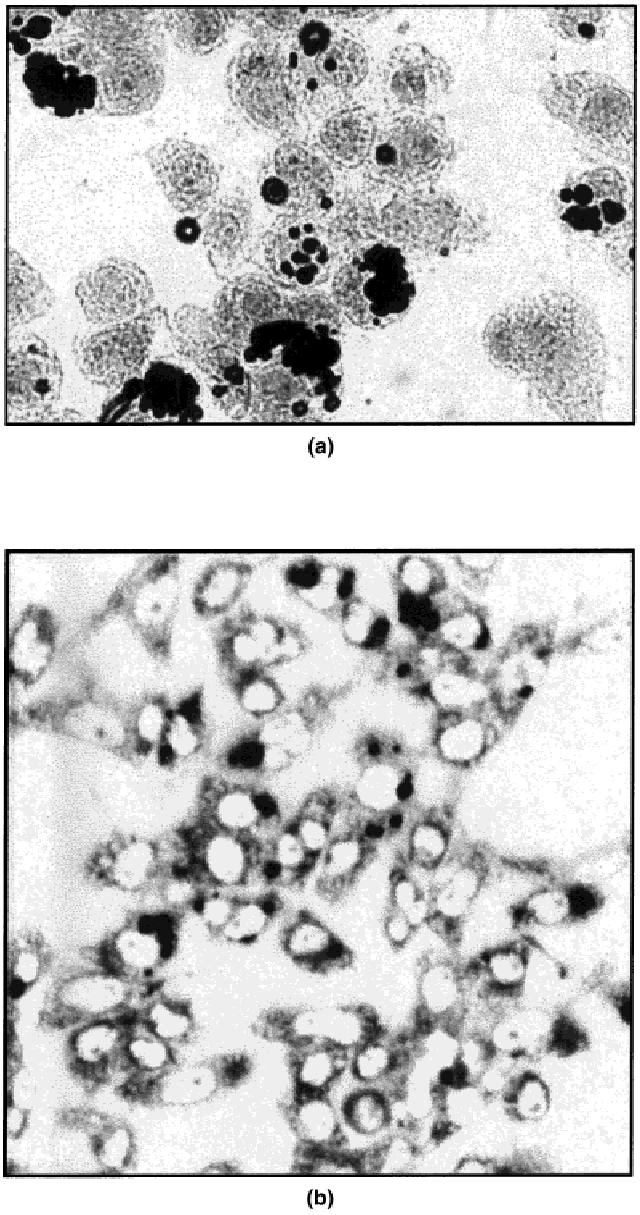

Figure 11. (a) Light and (b) confocal microscope images of IC-21 MØs phagocytosing CoCr particles on GEL-coated coverslips (after 24-h IMC experiment).

interaction of a macrophage cell line and particulates similar to wear debris from total hip prostheses surfaces.

Using a chamber and technique developed for this purpose in the course of these studies, IMC experiments showed significant heat flow rate differences for macrophages cultured with endotoxin (LPS positive control) or micron-sized particles (CoCr and HDPE) compared with cells cultured without such stimuli. Although statistical differences in CoCr particle treatments were not detected, there appeared to be a trend in the HDPE particle data that suggests differences between MØ response to clean and LPS-bound particles. Also, a statistical difference was not observed for MØs cultured with HDPE particles versus MØs cultured with CoCr particles.

The authors acknowledge Calorimetry Sciences Corporation, Provo, UT; Johnson & Johnson, Inc., Raynham, MA; Wright Medical Technology, Inc., Arlington, TN; and Smith & Nephew Orthopaedics, Memphis, TN.

References

1. Pappas MA. Biological response to particulate debris from nonmetallic orthopedic implants. In: Wise DL, Trantolo DJ, Altobelli D, editors. Human biomaterials applications. Totowa, NJ: Humana Press; 1996. p 115–130.
2. Amstutz HC, Campbell P, Kossovsky N, Clarke IC. Mechanism and clinical significance of wear debris-induced osteolysis. Clin Orthop 1992;276:7–18.
3. Jiranek WA, Machado M, Jasty M, Jevsevar D, Wolfe HJ, Goldring SR, Goldberg MJ, Harris WH. Productions of cytokines around loosened cemented acetabular components. J Bone Joint Surg 1993;75-A:863–879.
4. Horowitz SM, Purdon MA, Mechanisms of cellular recruitment in aseptic loosening of prosthetic joint implants. Calcif Tissue Int 1995;57:301–305.
5. Goldring MB, Goldring SR. Skeletal tissue response to cytokines. Clin Orthop 1990;258:245–268.
6. Willert HG, Bertram H, Buchhorn GH. Osteolysis in alloarthroplasty of the hip: The role of ultra-high molecular weight polyethylene wear particles. Clin Orthop 1990;258:95–121.
7. Howie DW, Manthey B, Hay S, Vernon-Roberts B. The synovial response to intra-articular injection in rats of polyethylene wear particles. Clin Orthop 1993;92:352–357.
8. Voronov I, Santerre JP, Hinek A, Callahan JW, Sandhu J, Boynton EL. Macrophage phagocytosis of polyethylene particulate *in vitro*. J Biomed Mater Res 1998;39:40–51.
9. Horowitz SM, Gonzales J. Effects of polyethylene on macrophages. J Orthop Res 1997;15:50–56.
10. Shanbhag A, Jacobs JJ, Black J, Galante JO, Glant TT. Human monocyte response to particulate biomaterials generated *in vivo* and *in vitro*. J Orthop Res 1995;13:792–801.
11. Haynes D, Rogers SD, Howie DW, Pearcy MJ, Vernon-Roberts B. Drug inhibition of the macrophage response to metal wear particles *in vitro*. Clin Orthop 1996;323:316–326.
12. Luchetti WT, Gonzales JB, Horowitz SM. A comparison of the inflammatory response to cobalt chrome and titanium particles. In: Proc 42nd Ann Meet Orthop Res Soc, Atlanta, GA; 1996. p 507.
13. Ikomi-Kumm J, Ljunggren L, Monti M. Thermochemical studies of surface-induced activation in human granulocytes *in vitro*. J Mater Sci Mater Med 1992;3:151–153.
14. Ikomi-Kumm J, Ljunggren L, Lund U, Monti M, Thysell H. Microcalorimetric evaluation of blood compatibility of hemodialysis membranes. Blood Purif 1991;9(4):177–181.
15. Van de Motter RR, Goldberg, VM, Niomiya J, Greenfield EM. Adherent endotoxin exists on orthopaedic implant wear surfaces and particles. In: Proc 45th Ann Meet Orthop Res Soc, Anaheim, CA; 1999. p 873.
16. Ragab A, Bi Y, Lavish A, Van de Motter RR, Goldberg VM, Greenfield EM. Stimulation of cytokine production by titanium wear particles is due to adherent endotoxin. In: Proc 44th Ann Meet Orthop Res Soc, New Orleans, LA; 1998. p 355.
17. Ralph P. Established cell lines as a source of functional macrophages. In: Herscowitz HB, editor. Manual of macrophage methodology: Collection, characterization, and function. New York: Marcel Dekker; 1981. p 51–59.
18. Simon LM, Robin ED, Phillips JR, Acevedo J, Axline SG, Theodore J. Enzymatic basis for bioenergetic differences of alveolar versus peritoneal macrophages and enzyme regulation by molecular O2. J Clin Invest 1977;59:443–448.
19. Butterick CJ, Williams DA, Boxer LA, Jersild RA, Mantich N, Higgins C, Baehner RL. Changes in energy metabolism, structure, and function in alveolar macrophages under anaerobic conditions. Br J Haematol 1981;48:523–532.
20. Glant T, Jacobs J. Response of three murine macrophage populations to particulate debris: Bone resorption in organ cultures. J Orthop Res 1994;12:720–731.
21. Oonishi H, Saito M, Kadoya Y. Wear of high-dose gamma irradiated polyethylene in total joint replacement: Long term radiological evaluation. In: Proc 44th Ann Meet Orthop Res Soc, New Orleans, LA; 1998. p 97.
22. Hahn DW, Wolfarth DL, Parks NL. Characterization of submicron polyethylene wear debris from synovial-fluid samples of revised knee replacements using a light-scattering technique. J Biomed Mater Res 1996;31:355–363.
23. Schmalzried TP, Campbell P, Schmitt AK, Brown IC, Amstutz HC. Shapes and dimensional characteristics of polyethylene wear particles generated *in vivo* by total knee replacements compared to total hip replacements. J Biomed Mater Res 1997; 38:203–210.
24. Shanbhag AS, Hasselman CT, Rubash HE. Technique for generating sub-micrometer ultra high molecular weight polyethylene particles. J Bone Joint Surg 1996;14:1000–1004.
25. Watson WC, Daniels AU, Charlebois SJ, Smith RA. Macrophages, particles and osteolysis: A microbial debris hypothesis. In: Trans Soc Biomater, Providence, RI; 1999. p 252.
26. Valdemarsson S, Ikomi-Kumm J, Monti M. Cell metabolic activity in acromegaly: A microcalorimetric study of lymphocyte metabolism. Acta Endocrinol 1990;122:422–426.
27. Ikomi-Kumm J, Monti M, Wadso I. Heat production in human blood lymphocytes: A methodological study. Scand J Clin Lab Invest 1984;44:745–752.
28. Schon A, Wadso I. Thermochemical characterization of T-lymphoma cells under non-growing conditions. Cytobios 1986; 48:195–205.
29. Valdermarsson S, Ikomi-Kumm J, Monti M. Thyroid hormones and thermogenesis: A microcalorimetric study of overall cell metabolism in lymphocytes from patients with different degrees of thyroid dysfunction. Acta Endocrinol 1990;123:155–160.
30. Parsson H, Nassberger L, Thorne J, Norgren L. Metabolic response of granulocytes and platelets to synthetic vascular grafts: Preliminary results with an *in vitro* technique. J Biomed Mater Res 1995;29:519–525.
31. Nassberger L, Jensen E, Monti M, Floren CH. Microcalorimetric investigation of metabolism in rat hepatocytes cultured on

microplates and in cell suspensions. Biochim Biophys Acta 1986;882:353–358.
32. Thoren SA, Monti M, Holma B. Heat conduction microcalorimetry of overall metabolism in rabbit alveolar macrophages in monolayers and in suspensions. Biochim Biophys Acta 1990; 1033:305–310.
33. Hoffner SE, Meredith RW, Kemp RB. Estimation of heat production by cultured cells in suspension using semi-automated flow microcalorimetry. Cytobios 1985;42:71–80.
34. Backman P, Kimura T, Schon A, Wadso I. Effects of pH-variations on the kinetics of growth and energy metabolism in cultured T-lymphoma cells: A microcalorimetric study. J Cell Physiol 1992;150:99–103.
35. Valdermarsson S, Ikomi-Kumm J, Monti M. Increased lymphocyte thermogenesis in hyperthyroid patients: Role of Na/K pump function—evaluation of aerobic/anaerobic metabolism. Acta Endocrinol 1992;126:291–295.
36. Valdemarsson S, Monti M. Increased ratio between anaerobic and aerobic metabolism in lymphocytes from hyperthyroid patients. Eur J Endocrinol 1994;130(3):276–280.
37. Loike JD, Silverstein SC, Sturtevant JM. Application of differential scanning microcalorimetry to the study of cellular processes: Heat production and glucose oxidation of murine macrophages. Proc Natl Acad Sci 1981;78:5958–5962.
38. Kemp RB. Importance of the calorimetric-respirometric ratio in studying intermediary metabolism of cultured mammalian cells. Thermochim Acta 1990;172:61–73.
39. Olsson SA, Monti M, Sorbris R, Nilsson-Ehle P. Adipocyte heat production before and after weight reduction by gastroplasty. Int J Obes 1986;10:99–105.
40. Gruwel ML, Alves C, Schrader J. Na(+)-K(+)-ATPase in endothelial cell energetics: 23Na nuclear magnetic resonance and calorimetry study. Am J Physiol 1995;268:351–358.
41. Okuda A, Kinura G. Contribution of growth factors to heat production by cultured rat fibroblasts. Tokai J Exp Clin Med 1990;15:363–367.
42. Ding JW, Nassberger L, Andersson R, Bengmark S. Macrophage phagocytic dysfunction and reduced metabolic response in experimental obstructive jaundice. Eur J Surg 1994;160:437–442.