

ISOTHERMAL MICROCALORIMETRY (IMC): A NEW IN-VITRO TECHNIQUE FOR DETERMINING BIOMATERIALS STABILITY & CELLULAR BIOCOMPATIBILITY

A.U. Daniels¹, D. Wirz¹, S.J. Charlebois², G. Lewis³

¹ *Laboratory for Orthopedic Biomechanics, Univ. of Basel, Basel, Switzerland*

² *Research Department, Zimmer, Inc., Warsaw IN, USA*

³ *Dept. of Mechanical Engineering, Univ. of Memphis, Memphis TN, USA*

INTRODUCTION: Some of the earliest scientific measurements were calorimetric—i.e. heat produced or absorbed during chemical reactions or changes of state. Bunsen (1811–1899) placed small animals on blocks of ice and used rate of melting as a measure of metabolic activity. Calorimetry has, of course, progressed enormously. However, not many biomaterials scientists are aware of the power of isothermal microcalorimetry (IMC) for quickly determining either rates of biomaterial degradation processes or magnitudes of cultured cell metabolic responses to biomaterials. This paper presents IMC advantages, illustrative studies, and possible future applications.

IMC ADVANTAGES: IMC has six attractive features for determining process kinetics and energetics. Sensitivity. A few grams of sample are usually sufficient for measuring even slow processes, such as oxidation of stable polymers. Rapidity. IMC can capture rate constants for slow processes in a few hours. Methods based on accumulation and quantitation of degradation products can require months. Simplicity. Studies of slow processes frequently can be carried out in sealed ampoules, with no need to monitor or replenish the environment, since there is often no significant consumption of reactants or accumulation of products. Universal Detection. Most rate monitoring techniques record a change in one property; e.g., pH, so the rate process must be understood in advance. IMC is a universal detector since heat is produced or consumed in all physico-chemical processes. Direct Determination of Kinetics & Energetics. Aggregate rates can be determined directly at one temperature using curve-fitting software to find a rate equation. Heat energy evolved or absorbed in transient processes (e.g., surface adsorption) can be obtained directly by integrating heat flow data. Re-Use of Specimens. If a slow process has been measured, the IMC specimen is little changed and thus available for other studies.

PREDICTING BIOMATERIALS STABILITY: IMC data are used routinely to quickly predict shelf life of solid pharmaceuticals. To our knowledge, IMC has not been used to study the stability of implant materials, other than in our recent work [1,2]. We have studied stability of UHMWPE, CaSO₄ bone void

filler, and acrylic bone cement powders in air and buffered saline. One major finding was that radiation sterilization increases the oxidation rate of UHMWPE 7X-10X compared to EtO, and the rate difference persists after ~9 years (!) of post-sterilization shelf storage and/or clinical TJA implantation. Oxidation embrittles UHMWPE.

MEASURING CULTURED CELL METABOLIC RESPONSES:

The first known IMC study [3] of cell metabolic response to biomaterials evaluated response of granulocytes to dialysis membranes. The order of difference in response to 4 different membrane materials correlated exactly with clinical studies of compromised granulocyte and leukocyte function. We used IMC to study response of transformed macrophages to TJA wear particles [4] (U.S. NIH-NIAMS grant 1R41-AR44581-01). The rate of metabolic heat generated by $\sim 1.25 \times 10^6$ cells in RPMI was $\sim 22 \mu\text{W}$ ($\sim 18 \text{ pW/cell}$). Exposure to $\sim 1.25 \times 10^6$ of 1-6 μm gel-trapped particles (either Co alloy or HDPE) raised heat flow by 15-20 μW . LPS was used separately as a positive control. Also, LPS further increased metabolic response when adsorbed on HDPE, but not on Co alloy.

SUMMARY: IMC is a uniquely sensitive, rapid, direct means for quantitating both biomaterials degradation rates and responses of cultured cells to biomaterials. Planned IMC studies include surface adsorption of cells and bioactive molecules.

REFERENCES: ¹Lewis G, Daniels AU (2003) Use of Isothermal Heat-Conduction Microcalorimetry (IHCMC) for the Evaluation of Synthetic Biomaterials, *JBMR* 66B:487-501. ²Charlebois SJ, Daniels AU, Lewis G (2003) Isothermal Microcalorimetry: An Analytical Technique for Assessing the Dynamic Chemical Stability of UHMWPE, *Biomaterials* 24:291–296. ³Ikomi-Kumm J, Ljunggren L, Lund U, Monti M, Thysell H (1991) Microcalorimetric Evaluation of Blood Compatibility of Hemodialysis Membranes. *Blood Purif.* 9:177-181. ⁴Charlebois SJ, Daniels AU, Smith RA (2002) Metabolic Heat Production as a Measure of Macrophage Response to Particles from Orthopaedic Implant Materials, *JBMR* 59: 166-175.