

# Development of Isothermal Microcalorimetry Methods for Microbiology and Human Cell Pathology at the Uni Basel Hospital

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## Background

How did an orthopedic biomechanics lab start doing IMC? A billion or so micron-size wear particles are generated by the articular surfaces of a standard clinical human hip replacement each year. In some patients, these particles elicit a persistent inflammatory response, eventually leading to bone absorption around the implant and clinical failure due to implant loosening. In 1996, Daniels (at U. Tennessee School of Medicine), wished to understand whether the response of the macrophages which drive the inflammatory process depended on the type of debris. He encountered Swedish papers showing that metabolic responses of living cells to materials could be measured with IMC. NIH and other funding was obtained, an instrument acquired, and an IMC method developed. The response of cultured macrophages to small particles did indeed differ for metal and polymeric hip implant materials [1]. IMC material stability studies were also done.

## Rapid IMC Detection of Microorganisms in Clinical Specimens

In 2004, now at LOB, Daniels again acquired an IMC instrument (TAM III, 6 microcalorimeters, 3 twin nanocalorimeters, Thermometric, Inc., Järfälla, Sweden) for both cultured cell and implant materials studies. In late 2004 LOB learned of a project in which removed implants were being sonicated to remove any attached microorganisms. The sonicate fluids were assessed for presence of microorganisms using conventional microbiology. Daniels suggested to the infectiologist (Trampuz) that rising IMC heat flow signals might be a rapid and sensitive means for detecting microorganisms in sonicates and other specimens, using a simple approach: monitoring heat flow from a sealed IMC ampoule containing a growth medium and the specimen.

In early 2005, detection in sonicates was successful, and another experiment also indicated that IMC had broad potential. As shown (Fig. 1) detection of growth of a typical microorganism in 3 ml of culture medium occurs in a few hours, even if the initial concentration is only 3 CFU! (LOB has subsequently developed a prototype program for analyzing heat flow curves to detect the onset of exponential growth.) A collaboration ensued. Other 2005 experiments showed that (a) heat flow growth curves in a given culture medium are different for different microorganisms, and (b) heat flow curves for a given microorganism are different in different media. This means IMC also has potential for identification of the type of microorganism present in a specimen.

Based on those experiments, additional funding was obtained, and a high-capacity IMC instrument (TAM III, 48 microcalorimeters) was acquired in late 2005. In 2006, studies were undertaken to explore IMC for rapid detection of microorganisms in blood, blood products and cerebrospinal fluid, all with promising results. In general IMC is potentially several times faster than conventional culture plate methods, and also simple and safe. Three medical dissertations resulted, and the blood product study has been published [2]. Current LOB studies focus on (a)

using IMC for rapid detection of MRSA (methicillin-resistant *Staph. aureus*) in specimens, using the effects on growth when antibiotics are added to the culture medium, and (b) use of pattern recognition--specimen responses to media arrays--as a potential means of identification.

### IMC Evaluation of Lymphocyte Proliferation in Response to Stimulating Agents

In early 2006, IMC's ability to monitor cultured cell proliferation was discussed with an immunologist (Regenass). Lymphocyte proliferation in response to stimulants is an important clinical measure of lymphocyte function. In the standard assay, harvested lymphocytes are placed in culture, a stimulating agent is added, and days later, radioactive thymidine is added. Thymidine uptake and consequent radioactivity of the specimen is roughly proportional to the number of lymphocytes present. We first compared IMC heat signals from sealed ampoules to the thymidine uptake protocol, in response to a general stimulant, phytohemagglutinin. Results were highly correlated (Fig. 2). In addition, IMC has the advantage of eliminating radioactivity and providing a continuous assessment of proliferation. Also, IMC only requires  $2.5 \times 10^5$  lymphocytes in 0.5 ml of medium. Surprisingly, proliferation goes on steadily for a week or more in a sealed ampoule at 37°C. In late 2006, modest internal funding was obtained. Current studies are assessing the ability of IMC to quantitate the response to more specific stimulants.

### IMC Determination of Heat of Adhesion of Bacteria to Surfaces

A microbiologist (Meyer) at the Dental Institute is interested in adherence of bacteria on dental surfaces. In 2006, we suggested that a sealed ampoule IMC method might be able to measure the heat involved in the adhesion process. With dental bacteria in media in which they do not grow, we found that heat flow differences increased in response to increases in glass surface area available for adhesion, suggesting the approach is feasible. The work is accepted for publication [3]. Current studies are aimed at simple ways to detect heat at earliest stages of adhesion.

### References

1. Charlebois SJ, Daniels AU, Smith RA (2002) Metabolic heat production as a measure of macrophage response to particles from orthopedic implant materials. *J. Biomed. Materials Res.* 59: 166-175.
2. Trampuz A, Salzmann S, Antheaume J, Daniels AU (accepted 2/2007) Microcalorimetry - a novel method for detection of microbial contamination in platelet products. *Transfusion.*
3. Hauser-Gerspach I, Scandiucci de Freitas P, Daniels AU, Meyer J (accepted 5/2007) Adhesion of streptococcus sanguinis to glass surfaces can be measured by isothermal microcalorimetry (IMC). *J. Biomed. Materials Res.- Part B.*

Fig. 1: Heat flow curves @ 37°C, *Staph. epi.* in soy broth

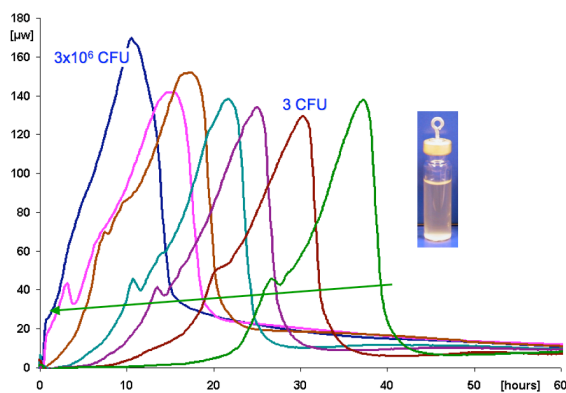


Fig. 2: Lymphocyte proliferation. IMC vs. thymidine uptake.

