

## PERSONAL RESEARCH STATEMENT 2008

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### VISION AND OVERVIEW:

Heat and mass transport in biomaterials is at the forefront of a number of new areas of knowledge and technology that are being used in the treatment of cardiovascular, cancer and neural disease. My work in this area focuses most heavily in biopreservation and thermal therapies, with an increasing interest in the fundamentals of thermal and mechanical properties, multi-scale imaging, and nanoparticle technology for disease assessment and treatment. My work has resulted in research that crosses disciplinary boundaries. This multidisciplinary breadth is reflected in my publication record in engineering (e.g. *ASME*, *ABME*, *AICHE*, *IEEE*), biology and biophysics (e.g. *J. of Applied Physiology*, *Cryobiology*, *Int. J. Hyperthermia*, *Biochimica et Biophysica Acta*) and translational and clinical journals (e.g. *Molecular Cancer Therapeutics*, *Endourology*, *Urology*, *J. Clin. Invest.*). This statement will describe ongoing work in biopreservation, micro- and macroscale thermal and mechanical properties, thermal therapies, nanotechnology and sickle cell disease characterization.

### BIOPRESERVATION:

Biopreservation encompasses both cryobiology and anhydrous biology. The goal is to prolong the "shelf life" of biomaterials in the chilled, frozen, vitrified (i.e. amorphous or glassy state), or dried state. The fruits of this discipline are used routinely in biomedical research, medicine, and industry. Recent breakthroughs include the preservation of tissues (i.e. veins, heart valves) by vitrification and human sperm, oocytes and blood by freezing. Broad applications include cell, blood, organ and tissue banking, and artificial or assisted reproduction, and drug, vaccine and food stabilization.

We are actively pursuing cryopreservation of mouse sperm and especially rat sperm, an important and developing transgenic rodent system. At the molecular scale we have introduced new measurements including differential scanning calorimetry (Devireddy et al. 1999, *Biol. Of Reproduction*) and Fourier transform infra-red spectroscopy (FTIR) to probe the state of water during freezing in and around both cells and their membranes. In particular, the measurement of macromolecular hydration (i.e. lipid and protein) during freezing shows for the first time a direct correlation between cellular dehydration and intracellular ice formation which destroys cells (Wolkers et al. 2007 *BBA*). By tracking molecular events (which is easier than cellular), this result promises even greater insight and control of freezing events in sperm (and other cells) to achieve a particular viability outcome from biopreservation.

At the tissue level, there is a transplantation crisis due to the demand exceeding the supply of viable tissue for transplantation in the United States. The development of preservation protocols to bank, store, and transport both donor and engineered tissues for transplantation and clinical use is one important approach to meeting the transplantation crisis (Han and Bischof 2004, *CPT*). My group has developed new low temperature microscopy and thermal analysis (differential scanning calorimetry – DSC) tools to measure the state of intracellular and extracellular water (and its movement) within both native and engineered tissues. We have also shown that cooling rate (which affects the formation of intra and extracellular ice) impacts both viability (i.e. alive vs. dead) of the cells within engineered tissue as well as the mechanical properties of the tissue (or graft) function. We are focused on preservation of cardiovascular and native organ (artery, liver, kidney) tissue slices. My goal is to control the ratio of crystalline ice and amorphous glass formation in tissues in order to increase post thaw viability and reduce adverse mechanical property changes (i.e. maintain the elasticity of an artery). One promising approach is the development of non-invasive imaging techniques (optical and clinical imaging technology – US, CT and MR) that track thermal and chemical changes as well as bulk phases (ice, glass and liquid) within heterogeneous tissues during preservation protocols (Bischof et al. 2007 *ABME*). This work was the first to use imaging (CT) to non-invasively measure these bulk phases in frozen tissues and show the potential of this technique for non-invasive quality control and assessment in frozen biomaterials.

## **MICRO AND MACROSCALE THERMAL AND MECHANICAL PROPERTIES:**

In all freezing protocols (for biopreservation or thermal therapies), the thermal history is critical to the preservation (or injury) outcome. The size, location and structure of the ice (or glass), is important in determining the outcome of a freezing protocol. This in turn is determined by the thermochemical environment during cooling. My group is currently investigating the microscale phases and thermal properties of frozen solutions and native tissues with application to both biopreservation and thermal therapy (Choi and Blschof 2007 *Int. J. or Heat and Mass Transfer*, Choi and Blschof 2008 *Cryobiology*). A relatively new area of interest in my lab focuses on the intersection of thermodynamic excursions with biomechanical property change. Our work and others in this evolving field of "thermobiomechanics," suggests that hydration, protein structure and cellular viability are all important in defining and maintaining biomechanical properties in functional tissues (i.e. cardiovascular). Our freezing work with arteries is some of the first to clearly demonstrate both bulk water loss and stiffening of the arteries post freeze thaw (Venkatasubramanian et al. 2006 *ABME*). Work is ongoing to extract the fundamental mechanisms (cellular, collagen ECM structure and water interactions) that affect these biomechanical changes during a thermal excursion.

## **THERMAL THERAPIES:**

Urology and cardiovascular surgery are two important surgical disciplines in which thermal therapies for treatment of benign prostatic hypertrophy (BPH), cancer (prostate and kidney), arterial disease, heart arrhythmias, and fibrillations and other diseases are actively under investigation. There are numerous other targets of heat and cold in the body such as the brain, spine, liver, and breast, such that most if not all of the surgical sub-disciplines now actively use one or more thermal therapies. One driving force for the use of thermal therapeutic probes are their minimally or non-invasive nature which allows focal and repeatable treatments of patients under local vs. general anesthesia. This has made treatment an option to a growing population of patients who 20 years ago would have been considered inoperable due to surgical risk. These probes are based on heating technology (radiofrequency, microwave, high intensity focused ultrasound, or laser) or cryogenic technology (cryosurgery). While the use of heat and cold in medicine has a long history, the precise thermal conditions and mechanisms that destroy various tissues within the body are still under investigation and a main goal of my program as reviewed in (Hoffman and Bischof, 2002 *Urology* and He and Bischof, 2003 *Crit. Rev. Biomed. Engin.*).

In cardiovascular disease such as restenosis, atrial fibrillation, tachycardias, arrhythmias, and even myocardial infarction, catheter-based cryo and heat treatments are being investigated for management or cure. We are currently investigating the use of cryosurgical protocols to treat: 1) restenosis in arterial targets (peripheral arteries) and 2) atrial fibrillation by using a pulmonary vein target. Porcine artery explants are being assessed for viability and mechanical property changes and *in vivo* work in both sheep and pig models are currently underway. We have shown that freezing has a definite impact on both viability as well as the mechanical state of the vessels post-freeze. The mechanisms whereby these changes occur are being assessed at the molecular, interstitial matrix (ECM), cellular, and whole tissue level with and without inflammatory stimulation (Venkatasubramanian et al. 2006 *ABME*, Balasubramanian et al. 2007 *ABME*).

## **OTHER BIOTRANSPORT RELATED RESEARCH PROJECTS:**

### ***Nanotechnology in Biomedical Applications:***

Our nanotechnology work has grown out of interest in thermal therapies and adjuvant (chemical additive) or drug delivery questions. While a relatively new focus for my program, these projects focus on the use of both magnetic and gold nanoparticles (10 – 100 nm size) for biomedical applications in which their movement, visualization or heating show promise for improved disease treatments. Possible *in vivo* applications of chemicals attached to magnetic nanoparticles for drug delivery and targeted heat treatments are being pursued. These magnetic nanoparticles can also be imaged with magnetic resonance (MR) and computed tomography (CT), directed by static magnetic fields, and heated inductively by radiofrequency (Kalambur et al. 2007 *Langmuir*). In addition, while TNF- $\alpha$  has been shown in my previous work (and by others) to be an excellent thermal therapeutic adjuvant (chemical which augments the injury), it can lead to systemic toxicity if given intravenously at too high a dose. Thus, another exciting *in vivo* application of nanotechnology is to avoid systemic toxicity of drugs such as TNF- $\alpha$  by attaching them to nanoparticles that can accumulate to cytotoxic

concentrations in the tumor. This has been achieved by our collaborators Cytimmune Sciences, Inc (Rockville, MD) in a gold nanodrug CYT-6091 (in Phase I Clinical Trial) which we have successfully used to destroy all cancer within rodents by thermal therapy (Goel et al. 2007 *Mol. Cancer Ther.*, Visaria et al. 2006 *Mol. Cancer Ther.*).

***Characterization of Blood Flow in Sickle Disease.***

My work in intravital microscopy and blood flow quantification in cancer has led to a collaboration investigating sickle disease with hematologists in the Department of Medicine at University of Minnesota. I am working to help these colleagues establish the connection between inflammation induced by a lack of oxygen (hypoxia) with blood flow changes including sickle crisis (flow stasis) in sickle disease. The dorsal skin fold chamber model implanted in transgenic sickle mice is used with optical Doppler velocimetry and image splitting to quantify blood flow (including stasis) after hypoxic insult followed by reoxygenation. In particular, we have documented both inflammation (activation and white blood cell rolling) as well as transient stasis in the microvasculature of transgenic sickle mice after hypoxia followed by reoxygenation and reperfusion. In addition, we have shown mitigation of these effects with various anti-inflammatory agents. This is significant as sickle disease affects over 70,000 patients a year in the U.S. alone and is only now being viewed as an inflammatory disease (Kalambur et al. 2004 *Am. J. Hematology*, Belcher et al. 2005 *Am. J. Phys.*).