AN IN VITRO CRYOSURGERY MODEL USING AN ENGINEERED ARTIFICIAL TISSUE

Bumsoo Han (1), Rebecca J. Volovsek (2), Mary E. Grim (2), Victor H. Barocas (2) and John C. Bischof (1, 2, 3)

(1) Department of Mechanical Engineering
University of Minnesota
Minneapolis, MN

(2) Department of Biomedical Engineering
University of Minnesota
Minneapolis, MN

(3) Department of Urologic Surgery
University of Minnesota
Minneapolis, MN

INTRODUCTION

Cryosurgery is a minimally invasive surgery technique in which unwanted or malignant tissue is destroyed by freezing. Due to its minimally invasive characteristics and recent advances in monitoring technology during a surgery, cryosurgery is emerging as a promising treatment modality of various cancers including prostate, liver and breast cancers. It is still necessary, however, to understand the mechanisms of freezing induced tumor destruction and thermal conditions for tumor destruction, in order to improve the efficacy of cryosurgery.

To investigate the mechanisms and thermal conditions, several different cryosurgery models have been developed including cell suspension, cell monolayer on a substrate, in vitro native tumor and in vivo tumor. Even though an in vivo tumor system is the most realistic experimental model for cryosurgery, it is very difficult to control and analyze the results since so many parameters are intermingled. Contrary to in vivo systems, cell suspensions and cell monolayers are easy to control, but sometimes oversimplify cryosurgery situations. Therefore, it is necessary to develop an experimental cryosurgery model which is controllable but still maintains as much real tissue characteristics as possible (i.e. cell-cell contact and cell- cell-extracellular matrix (ECM) contact).

In the present study, an in vitro cryosurgery model was developed with an engineered artificial AT-1 tumor tissue. Comparing with cell suspensions and cell monolayers, the artificial AT-1 tumor system can investigate the effect of ECM interaction and cell based healing response at different time points after cryosurgery, but it is significantly easier to control and analyze than in vivo systems. The developed model was also examined through an in vitro cryosurgery situation.

MATERIALS AND METHODS

Engineered Artificial Tumor Tissue

Engineered tumor tissues were composed of collagen fibers with AT-1 tumor cells entrapped in them. AT-1 collagen gels were prepared with reagents at 4°C by a recipe consisting of individual media components, AT-1 tumor cells, and an acidic collagen solution (Organogenesis, Canton, MA). The final concentration of collagen and cells in the constructs were 1 mg/ml and 100-300,000/ml, respectively. Solutions will be transferred to 15 x 60 mm petri dishes and allowed to crosslink at 37°C for 1 hr. Samples were then flooded with culture medium and placed back into culture for 3-7 days before cryosurgery experiments.

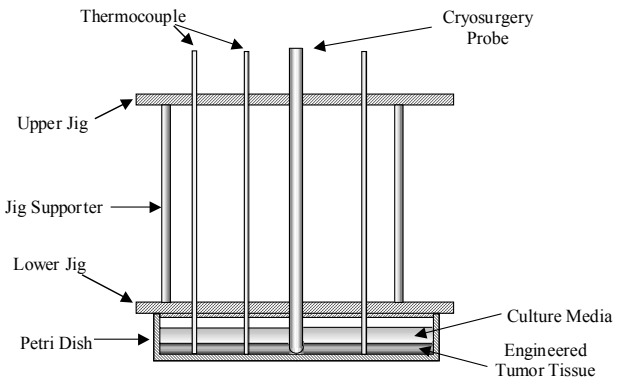

Figure 1: Schematic diagram of cryosurgery jig

Cryosurgery Setup

A schematic diagram of the cryosurgery setup is shown in Figure 1. A plastic jig held a 3.0 mm diameter, argon-driven cryoprobe (Endocare, CA) in the center of the gel, perpendicular to the petri dish. Type-T thermocouples were inserted through the jig and into the gel at multiple distances from the center of the cryoprobe. The cryoprobe target temperature (to -120°C) and freeze time (up to 15 min) were computer-controlled. Samples were allowed to passively thaw (21°C). Continuous temperature measurements from the thermocouples during freeze/thaw were recorded on a Fluke Hydra DataLogger (Dytec

Instruments, Bloomington, MN) and transferred to a PC for data reduction, graphing and analysis. Each sample was then flooded with 5 ml of medium and placed back into 37°C culture for 0-3 days before injury assessment.

Freezing Injury Assay

Following the in vitro cryosurgery, artificial tissues were assayed for viability immediately and up to 3 days later. Tissue was scored after a 30 minute incubation at 37°C with 9 µM Hoechst 33342 and 16 µM ethidium homodimer-1 (Molecular Probes). After staining, each tissue was washed in HBSS and analyzed at 200X with a fluorescent microscope (Olympus). Each radial distance of the gel corresponding to thermocouple placement during the surgery was assayed. The total number of cells in each field was determined by counting Hoechst 33342 positive nuclei; the number of non-viable cells in the same field was determined by counting the ethidium homodimer-1 positive cells. All samples were assayed in 4-6 replicates and the resulting values normalized relative to control viability.

Heat Transfer Modeling

The measured temperature data were compared with one dimensional transient conduction model. The governing equation will be

$$\frac{\partial}{\partial t}(\rho h) = \frac{1}{r}\left(kr\frac{\partial T}{\partial r}\right) \qquad (1)$$

where ρ is density, h is specific enthalpy, t is time, r is a spatial coordinate, k is thermal conductivity, and T is temperature. Equation (1) was numerically solved using an elliptic enthalpy method. The details about the numerical scheme were reviewed in [1]. The corresponding boundary conditions were obtained from the cryosurgery jig geometry and measured cryoprobe temperature.

RESULTS AND DISCUSSION

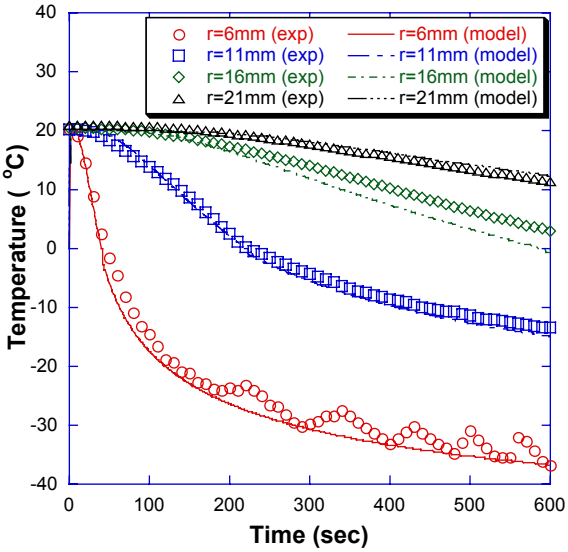

Figure 2: Comparison of measured and simulated temperature history during a cryosurgery

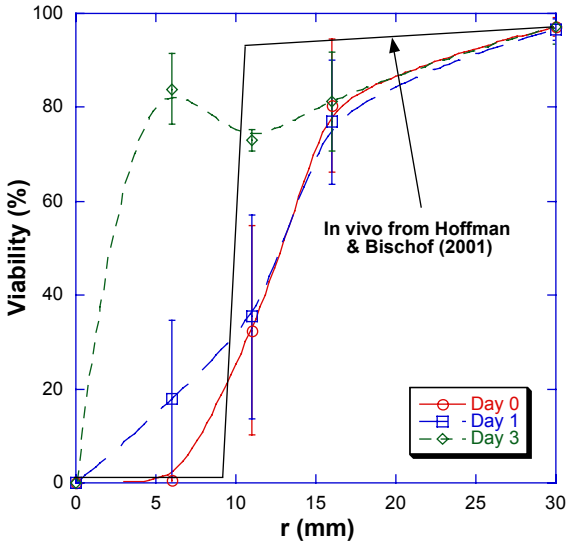

Figure 3: Viability of tissue engineered AT-1 tumor tissue after cryosurgery

Thermal History

Measured temperature variations at several different locations are compared with numerically simulated temperatures in Figure 2. Generally speaking, the developed numerical model predicts the thermal history during freezing very well. The small peaks in measured temperature at r=6mm are due to the artifact from the cryogen pumping in the cryosurgery probe. The temperatures at 6 and 11 mm show their slope change around 0°C due to ice formation. Based on the thermal histories, the artificial tumor tissues were frozen where r is smaller than 11 mm, but the region around r=11mm experiences very mild freezing condition.

Post-thaw Viability

Post-thaw viabilities at several locations at three different time points after the surgery (immediate, 1 and 3 days after) were shown in Figure 3. At Day 0, most of tumor cells were dead within the region close to the cryosurgery probe (≤6 mm), and the viability increases gradually in radial direction. However, this trend is not valid at in vivo systems. Based on the observation in [2], in vivo cryoinjury is abrupt rather than gradual change, which implies other injury mechanism – vasculature injury. At Day 1 and 3, viability of partially injured region (between 6 and 11 mm) increases due to healing response of the tissue.

ACKNOWLEDGEMENT

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