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Green Fluorescent Protein as an Indicator of Cryoinjury in Tissues

ADAM B. SLADE,[1] LORENZO A. MARTÍNEZ-SUÁSTEGUI,[2] FLORIAN VIÉ,[3] and GUILLERMO AGUILAR[1,4]

[1]Department of Mechanical Engineering, University of California Riverside, Riverside, CA 92521, USA; [2]ESIME Azcapotzalco, Instituto Politécnico Nacional, Avenida de las Granjas No. 682, Colonia Santa Catarina, Delegación Azcapotzalco, Mexico, Distrito Federal 02250, Mexico; [3]Department of Nuclear Engineering, National Graduate School of Engineering and Research Center of Caen (ENSICAEN), 6, Boulevard du Maréchal Juin CS 45053, 14050 Caen Cedex 04, France; and [4]Department of Bioengineering, University of California Riverside, Riverside, CA 92521, USA

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Abstract—The fluorescence intensity of Green Fluorescent Protein (GFP) has previously been demonstrated to be an accurate indicator of cellular viability following cryoinsult in individual GFP-transfected cells. In an attempt to ascertain whether GFP fluorescence intensity may also be used as a viability indicator following cryogenic insults in whole tissues, this study examines the transient fluorescence intensity of GFP-transfected mouse hepatic tissue *ex vivo* following cryoinsult. The observed trends are compared with diffusion-based models. It was observed that the fluorescence intensity of the exposed tissues exhibited slow exponential decay, while the solution in which the tissues were placed inversely gained fluorescence. This slow decay (~3 h) is in contrast to the rapidly diminished fluorescence intensity (seconds) seen in GFP-cell cultures following cryoinsult. These trends suggest that mass diffusion of GFP in the interstitial space, and ultimately into the surrounding medium, is the primary mechanism which determines the fluorescence loss in cryoinjured tissues. These results suggest GFP-transfected tissues may be effectively used as indicators of cryoinjury, and hence viability, following hypothermal insult provided that a sufficiently long incubation is held before observation. It was found that a meaningful observation (15% reduction in fluorescence) could be made three hours subsequent to cryoinjury for the tissues used in this study.

Keywords—Cryoinsult, Cryosurgery, Diffusion, Hyperthermia, Hypothermia, Fluorescence, Laser, Viability.

INTRODUCTION

Cryosurgery—the precise application of cryogenic temperatures—is often used to selectively devitalize cancerous or precancerous tumors, arresting its propagation through healthy tissue. These low temperatures serve to rupture the plasma cell membranes, or to dehydrate the cells as to render their vitality compromised. To determine the success of a cryosurgical protocol, it is of critical importance that the tissues cryogenically treated, as well as the surrounding tissues, be evaluated for their viability. Current viability protocols require the tissue to be excised from the treatment site and examined *in vitro*.[9,17] These procedures are invasive and provide only information traceable to the moment in time when the biopsy was obtained. Non-invasive viability assays can be utilized to determine cellular viability, but require the addition of chemical tracers (which are often toxic or phototoxic) to the living tissue.[13] While viability can be determined optically without tracers, this is done on a cell-to-cell basis,[4] and assessing the viability of a whole tissue would not be feasible using those methods.

Green Fluorescent Protein (GFP) is a protein derived from jellyfish which can be and has been transposed into the genome of a myriad of organisms. It has the effect of causing transfected tissues to fluoresce green when illuminated with blue light, and has recently found use as a viability assay in cell cultures.[3] GFP has the advantage over other viability assays in that it can be examined *in situ*, and can also be continuously monitored to examine the transient viability of the cells using optical imaging, thereby making it an ideal indicator of animal tissue viability in research protocols.

The GFP molecule has a tight cylindrical barrel shape, which protects it from a moderate range of thermal trauma,[18] however, at sufficiently elevated temperatures (> 50 °C) its structure becomes "unwound",

Address correspondence to Guillermo Aguilar, Department of Mechanical Engineering, University of California Riverside, Riverside, CA 92521, USA. Electronic mail: aslade@engr.ucr.edu, lamartinezs@ipn.mx, gaguilar@engr.ucr.edu