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Laser-Assisted Cryosurgery in *ex vivo* Mice Hepatic Tissue: Viability Assays Using Green Fluorescent Protein

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Abstract—An experimental investigation is carried out to develop a novel approach to cryosurgery, where laser heating counteracts tissue freezing to better confine damage to the targeted cancerous tissue within a lethal low-temperature isothermal boundary—an approach we refer to as laser-assisted cryosurgery (LAC). The advantage of this procedure relative to conventional cryosurgery assisted with urethral warmers or cryoheaters is that laser heating provides volumetric rather than superficial heating, which leads to deeper penetration, more homogeneous tissue protection and better demarcation of the destructive freezing effect to a well-defined targeted volume. Tissue viability assays are performed using green fluorescence protein (GFP) as a viability marker and correlated with temperature history after performing LAC procedures on *ex vivo* mice hepatic tissue. The limit for cell denaturation at the irradiated surface predicted by GFP analysis is further confirmed using reverse transcription polymerase chain reaction (RT-PCR). In addition, the correlation between GFP fluorescence and cell viability and loss of GFP fluorescence in non-viable cells has been tested and validated by histological analysis using a standard cell viability measuring method (hematoxylin and eosin staining). Analysis of our experimental measurements show that reproducible thermal gradients (of 236 °C/cm) and predictable tissue necrosis can be reliably produced by LAC without exceeding temperature thresholds for cell denaturation (of $T_{surf} \approx 48$ °C) beyond preset tissue boundaries (with resolution of 0.1 °C/mm). The results have shown the feasibility of controlling temperatures at specified tissue locations to prevent hyperthermal or freezing damage.

Keywords—Cryoablation, Prostate cancer, RT-PCR.

NOMENCLATURE

d	Distance (mm)
SD	Standard deviation
t	Time (s)
T	Temperature (°C)
TC	Thermocouple

Greek Letters

δ	Thickness of protection layer (mm)
μ_a	Absorption coefficient (mm^{-1})

Subscripts

Amb	Ambient
Cryo	Cryoprobe
Surf	Surface
Th	Threshold

INTRODUCTION

Cryosurgery is the infliction of a lethal freezing injury achieved by the contact of one or more metallic probes at low subzero temperatures with a target tumor tissue. Prostate cryosurgery was the first minimally invasive cryosurgical procedure to pass from the experimental stage and become a routine surgical treatment.[25] While the main objective during a cryosurgical procedure is to ensure tissue destruction within the cryolesion, the greatest challenge is on how to spare the surrounding healthy tissues from cryoablation. In order to overcome this difficulty and with the aid of recent technological developments, the total number of cryoprobes used during a cryosurgical procedure has been increased because of a considerable

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