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CRYOSURGERY FOR BREAST MALIGNANCIES: APPARATUS AND TECHNIQUES

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INTRODUCTION

The treatment of breast cancer has evolved from the time of mutilation and ignorance in the middle ages, to one of breast conserving management and an intense study and understanding of the biological mechanisms driving tumor cells. As the treatment is directed to the cellular and sub-cellular level, breast conserving surgical procedures take on a more important role. Recent published results from neoadjuvant trials indicate a decrease in tumor size in 80% of patients and a modest increase in conversion from mastectomy to lumpectomy. By 2010 AD, it is estimated that 50% of all new breast cancers discovered will be less than 10 mm in diameter (Cady et al., 1996), which represents 90,000 patients. Standard surgical treatment would require an open segment resection, an operating room, anesthesia, cosmetic concerns and substantial cost. Add to this the number of patients who require segmental resection following complete clinical or pathological response following neoadjuvant chemotherapy, and the cost increases.

An alternative method of tumor removal or destruction for small malignancies is needed to complete the biological assault on breast cancer. Cryosurgery may be one of these alternative means. Cryosurgery has been used successfully for more than three decades to treat benign and malignant neoplasms. To date, there is one reported case of primary breast cancer treatment with cryotherapy (Staren et al., 1997), which was followed up with ultrasound-guided biopsy, and which was found negative for malignancy 12 weeks post-cryosurgery. Cryotherapy carries many benefits in addition to the attractive concept of minimally invasive surgery. Low temperatures generate anaesthetic effect. Hemorrhage is reduced due to thrombosis of small blood vessels. Cryotherapy may cause stimulation of the body's immune system, which additionally augments local tumor destruction and may also induce a response in metastatic tumor sites (Suzuki, 1995).

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With multiple treatments such as neoadjuvant therapy, hormone therapy, and radiation, which have the ability to downsize primary cancers and treat small cancers, lumpectomy may be increasingly used. Current diagnostic imaging trends are increasingly detecting small cancers (≤1 cm). The minimization of surgical intervention to compliment these trends is a natural progression of technology and understanding of the biological processes involved.

Our ongoing research program to evaluate cryosurgery in the breast is comprised of several phases: (i) development of a miniaturized cryoprobe and a cryodevice for minimally invasive breast cryosurgery; (ii) validation testing of the cryoprobe and device *in vivo*; (iii) development of a technique to evaluate the injury associated with cryotreatment of the breast; (iv) comparison of the ultrasound imaged "ice-ball" *in vivo* with the resulting cryoinjury immediately post-cryosurgery; and, (v) long-term follow-up post-cryosurgery in a recently-pregnant sheep breast model. The work to date is part of this report.

Keywords: Cryosurgery, Breast, Animal Model, Cryoneedle, MRI, Mammography, Ultrasound

CRYOSURGICAL APPARATUS

The cryoprobe is designed for application in breast tumors; however, it can be used for the treatment of other tumors. The cryoprobe consists of three main components: a cryoneedle, a thermal insulation shell, and a protection tube, which are assembled during and as a part of the cryosurgical procedure. The cryoneedle has a unique U shape configuration and a sharp pointed tip, which is made of two adjacent 1.15 mm stainless steel tubes (similar in diameter to a 18.5-gage hypodermic needle), Fig. 1. The cryoneedles are designed to have an active length in the range of 10 and 150 mm. A length range of 20 to 30 mm has been used in the current research. The cryoprobe is fed by a

portable liquid nitrogen container (1.9*l*). The liquid nitrogen container is pressurized by compressed air at a very low pressure level (30 psi). Typical variation of temperature at the cryoprobe outer surface can be achieved at maximal cooling power: 37°C at the initiation of the procedure, -55°C after 15 sec, -82.5°C after 30 sec, -107.5°C after 45 sec, -116.5°C after 90 sec, and -140°C when approaching steady state. This cryoprobe and cooling protocol generate an average frozen region diameter of 22.3 mm within 5 min of operation in sheep breast tissues (n=21). This cryosurgical device has a high thermal efficiency (43%). The experimental setup is described in detail by Rabin et al. (1997a).

The cryoprobe design allows for application by either stereotactic or ultrasound placement. The cryoneedle can be used with a stereotactic device since the cryoneedle dimensions are compatible with the commonly used biopsy needle.

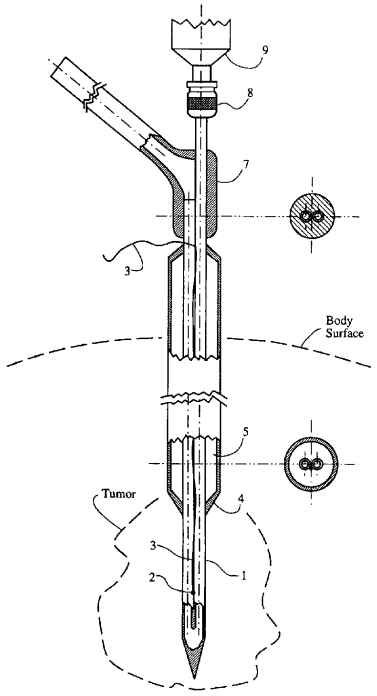

Figure 1: Schematic illustration of the cryoprobe (Rabin et al., 1997a)

VALIDATION OF THE CRYODEVICE

In vivo pilot experiments have been performed in order to examine the freezing ability of the new cryodevice and the extent of cryoinjury. Cryosurgical experimentation was conducted in the skeletal muscle of pig, pig liver, sheep liver, and in sheep breast. The cryoprocedure was monitored by Doppler ultrasound. Average frozen region diameters, as measured by ultrasound, were 25 and 40 mm after 10 minutes, using cryoprobes with a cross-section of l.3×2.6 mm and 2.2×4.4 mm, respectively. Histological studies, immediately post-cryosurgery, show severe cryoinjury regardless of the animal model (Rabin et al., 1997b). While the current validation testing concerns the immediate injury post-cryosurgery, cryoinjury may enhance up to a few days post-cryosurgery (Rabin, et al., 1999). Histological studies show that cryoinjury occurs also in the surroundings of the cryotreated tissue, outside of the frozen/thawed region as was observed via ultrasound. Vascular stasis as a widely recognized component of tissue injury post-freezing, such as frostbite, may contribute to the larger area of infarction when compared with the estimated size of the frozen region detected by ultrasound.

In the breast cases, results indicate that: the frozen region growth decreases with increase in breast size; the frozen region growth is faster in areas of fibrous breast tissue and slower in areas of large ducts and high fluid content. Results further indicate that ultrasound measurements may under estimate the area of cryodestruction when compared with histological measurements.

THE ANIMAL MODEL FOR BREAST CRYOSURGERY

The animal model chosen for the current study is a recently pregnant sheep breast model, 8 to 12 weeks post-lambing, and at least 4 weeks post-lactation. All animals were about 5 years old, after 5 deliveries (once a year), having a body weight in the range of 40 to 80 kg. Under these conditions, the sheep breast is similar in size and structure to the human breast. The animal model is required to be similar in size to the human breast from the following reasons: (i) there needs to be large enough volume of untreated tissue surrounding the cryoinjured site because the uninjured tissue surrounding the cryotreated site is expected to take a role in the recovery process; and, (ii) large size is needed to make imaging practical. Note that the sheep breast losses volume significantly in the long-term post-lactation.

All cryoprocedures were performed in areas of dense breast tissue fibers and as far as possible from the dilated breast ducts, where the location of the dense breast ducts network is close to the skin and under the nipple. Identification of the different areas of the breast and monitoring the ice-ball formation were performed using a Doppler ultrasound

The authors are not aware of any tumor model for sheep breast nor of any other tumor model of large animals which are similar in size and structure to the human's breast and, therefore, the cryotreatment of healthy breast tissues is a choice of practice.

EVALUATION OF THE CRYOINJURY

Three tissue fixation protocols have been tested to study the feasibility of preserving the entire breast for both macroscopic and microscopic examination, in order to determine the most practical method of examining the early pathologic effects of the cryotreatment (Rabin et al., 1998). In the first protocol, the whole specimens were immersed in 10% natural buffered formaldehyde for 48 h. The breasts were then bisected along the cryoneedle track and each half was immersed again in formaldehyde for a few weeks. In the second protocol, the breasts were bisected prior to fixation. The bisected specimens were subdivided into three parts as parallel as possible to the cryoneedle track. Parts 1 and 2 were separated from each other by dividing them along the cryoneedle track. Part 3 represented essentially normal tissue. Each of the parts was immersed in 10% natural buffered formaldehyde for a few weeks.

Perfusion with formaldehyde *in situ* was applied in the third protocol. All the major veins leading from the breasts were exposed and ligated about 15 min after thawing. The two major arteries leading to the breasts were exposed, cannulated and connected to a Harvard syringe pump, via a T-connector. The breasts were perfused with 150 *ml* of the

vital dye 2,3,5-triphenyltetrazolium chloride (TTC), 2% in phosphate-buffered saline, at a rate of 2.7 *ml*/min, followed by 50 ml of 10% natural buffered formaldehyde at a rate of 3.3 *ml*/min. The animals were maintained at 37°C throughout the procedure for optimal histochemical enzyme reduction in the tissue. The breasts were immediately excised and immersed in the same formaldehyde solution for 24 h. The specimens were then bisected along the cryoneedle track, the color of the cut surface was observed, and the specimens returned to the formaldehyde solution for a few weeks (Fig. 2).

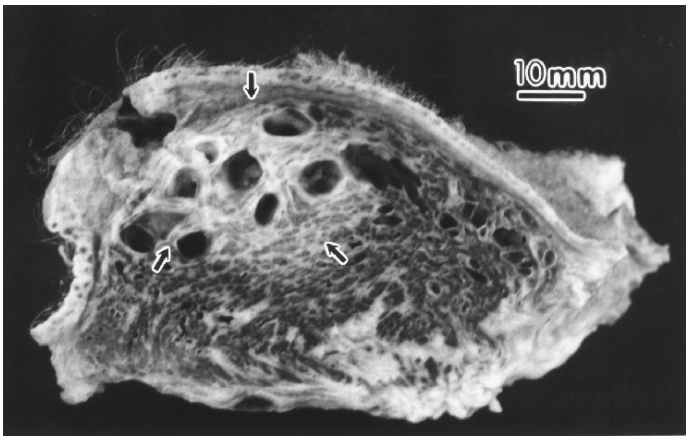

Figure 2: Macro cross-section view of a cryotreated breast specimen, which was perfused by 2,3,5-triphenyltetrazolium chloride (TTC) stain and 10% buffered formaldehyde. The cryotreated region appears pink (indicated by arrows), while the surrounding healthy tissues have a dark-red color, due to the staining of viable cells by the TTC. Note the large duct of the recently lactated ducts (8 weeks post-lambing). Bar length is 10 mm (top right). (Rabin et al., 1998)

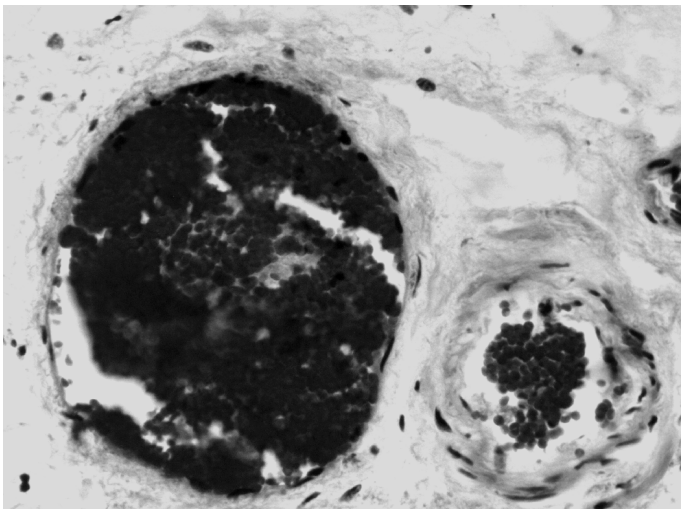

Figure 3: High power magnification shows a vein (left) and an artery (right) congested with blood and thrombosis from the center area of the cryotreated region. (Rabin et al., 1998)

Blocks of tissue, from the entire cryotreated region, were submitted for standard histological examination by light microscopy using hematoxylin & eosin stains (H&E). The cryotreated area was identified by decoloration of the tissue in the first two protocols and by staining in the third one.

Perfusion with formaldehyde *in situ* appeared to produce the best results among the three fixation protocols presented above: the specimen did not deform, no degeneration was observed, it was easy to trace the cryoneedle track, and it was easy to measure the cryotreated region.

Perfusion of a vital stain for viability testing *in situ* is an important technique in order to correlate the temperature field with cellular destruction around the cryoneedle. This technique enables evaluation of the extent of destruction in different areas of the ice-ball and avoids the difficulty in tracing the location and orientation of the tissue blocks which are processed for routine histological examination, as is the case in the ordinary procedure of stain diffusion in small tissue blocks. The TTC technique revealed less viable cells in the pink area which indicates the main cryodestructed region. This means that immediately after thawing, some cells appear to be viable in the main cryotreated region. The pink color immediately after thawing is more likely due to the congestion of red blood cells in the area (because this area turned brown as blood does with fixation), while the unfrozen region remained red. When using TTC for viability testing, it is assumed that TTC stains viable cells on the basis of the presence of respiratory enzymes; however, unstained tissue does not necessarily mean all cells are permanently damaged.

EVALUATION OF CRYOINJURY IMMEDIATELY POST-CRYOSURGERY

From ultrasound imaging, an under estimation of the cryotreated region in the range of 0.3 to 5.3 mm in the breast tissue was noted (Rabin et al., 1998). It is emphasized that the accuracy in measurements via enlarged ultrasound images (×5) is at least 5 times higher than that using a simple ruler, as was done in the histological study. Furthermore, the frozen region edge appears much sharper via ultrasound image than the edge of the cryotreated region, as it appeared during the histological examination. This difference is affected by many factors such as the biological response to low temperatures during thawing, and the tissue volume changes in the fixation process. For example, vasodilatation and congestion with blood are normal mammalian responses to tissue injury, in the injured region and in the periphery around the injured region.

The observation that the ultrasound-imaged "ice-ball" appears smaller than the resulted cryoinjury may partly be related to the physical principles of ultrasound imaging, where the temperature of the imaged freezing front is actually not known. The freezing front temperature is sometimes speculated to be the point at which pure water ice crystals start to form in equilibrium, i.e. 0°C. Assuming body solutions behave like an NaCl solution, the ultrasound imaged front can be somewhere between -22°C and 0°C (the phase transition temperature range). Furthermore, freezing can be suspended down to the homogeneous nucleation point in some cases, i.e. -39.2°C. An error of 20°C in defining the freezing front temperature can easily lead to an error in 2 mm in estimating the radius of the freezing front location (Rabin, 1998). Ultrasound imaging and thermocouples could be combined in order to verify the actual temperature of the ultrasound imaged interface. Unfortunately, this is not feasible due to the high uncertainty in temperature measurements using thermocouples during cryosurgery, an

uncertainty level which can easily reach the typical temperature range of phase transition (Rabin, 1998).

LONG-TERM FOLLOW-UP POST-CRYOSURGERY

A total of 20 recently-pregnant sheep, 8 to 12 weeks post-lambing, and at least 4 weeks post-lactation, were studied in six groups (Rabin et al., 1999).

The first group included 2 animals which were sacrificed immediately after a single cycle cryoprocedure in each of the two breasts. The second group included 3 animals which were followed-up for 7 days post-cryosurgery. Each animal was cryotreated with a single cycle cryoprocedure in one breast, while the other breast was taken as a control. Animals of the third, fourth, fifth, and sixth groups were followed-up for 1, 2, 3.5, and 5 months, respectively. A single cycle procedure was applied to one of the breasts of each animal. One half of the animals of the 1 to 5 months follow-up groups were used to compare a single cycle cryoprocedure in one breast with a three-cycle cryoprocedure in the other breast, which included 1 animal in each of the 1 and 2 months follow-up groups, and 2 animals in each of the 3.5 and 5 months follow-up groups. The remaining animals were used to compare the scar tissue developed as a result of a surgical excision with that developed after a single cycle cryoprocedure, which included 1 animal in the 3.5 months follow-up group, and 2 animals in each of the 3.5 and 5 months follow-up groups.

The surgical excision procedure included incision in the skin to a length of 15 to 20 mm, followed by excision of tissue with an average diameter of 10 to 15 mm Excision was performed with electrocautery.

All cryoprocedures were performed in areas of dense breast tissue and away from breast ducts. Identification of the different areas of the breast and monitoring the ice-ball formation were performed using a Doppler ultrasound (7 MHz linear array transducer). Ultrasound was also applied as a routine when the animals were sacrificed, in an effort to identify the site of cryotreatment.

MRI and mammography were applied about every 4 weeks in an effort to identify the sites of cryotreatment. The MRI was performed routinely on animals from the 5 months follow-up group, under general anesthesia, with animals on their back, in a Siemens© MRI unit with 1.5 Tesla strength. Mammography was performed on a larger number of animals from all follow-up groups, with 26 kV and between 56 and 63 MAS, in a Siemens© mammography unit. Mammography was performed *in vivo* under general anesthesia, where the animals were lying on their side. Also mammography was performed on the breast specimens immediately after harvesting.

Breast specimens were prepared for histological analysis by perfusion of the vital stain 2,3,5-triphenyltetrazolium chloride (TTC) followed by perfusion of formaldehyde *in situ*, as described above. Representative blocks of tissue from the cryotreated region were submitted for standard histological examination by light microscopy using hematoxylin & eosin stains (H&E), and with Masson's trichrome stain (Fig. 4). The pathological observations included microscopic assessment of cellular and vascular injury in the immediate post-cryosurgery period, as well as necrosis at one week. Glandular epithelial changes were assessed in all periods of observation. Reparative changes related to the development of scar tissue regeneration of epithelium were assessed at one month and later.

Comparison of ultrasound images and histology results indicate that, a conservative application of the cryosurgical device developed for the current study in breast tissues suggests 5 mm safety margins in an ultrasound monitored cryoprocedure. It follows that a target tumor diameter of 10 mm requires an ice-ball of 20 mm, which can be easily achieved within less than 5 minutes using the new cryosurgical device.

The cryoinjured region at 5 months post-cryosurgery is about one half the diameter of the imaged frozen region during the cryoprocedure. The reduction in cryoinjured region size with time is the result of: (i) the contraction of the scar tissue within the area of injury as the scar develops; (ii) the post-injury healing process of the tissue; and (iii) the natural reduction of the sheep breast with time post-lambing.

The cryotreatment site in the sheep breast model cannot be identified up to 5 months post-cryosurgery by means of ultrasound, mammography, or MRI. Using these standard imaging techniques, it is highly unlikely that scar formation will be misinterpreted as tumor or recurrence.

The cryoprocedure produces an immediate injury which is characterized by cellular degeneration with vacuolization of the cytoplasm and loss of cellular and nuclear detail. This injury is associated with vascular congestion with red blood cells and edema (Fig. 3). There is no gross or microscopic difference between lesions that have been subjected to one versus three freeze/thaw cycles. Under either cryosurgical protocol, there is a main cryoinjured region that has uniform destruction of epithelium and healing scar formation, and a transition zone of damaged lobules without acini, which are surrounded by healthy tissue. The surgical excision site forms a denser scar over the same time period, which suggests that the injury from cryosurgery recovers at a slower rate.

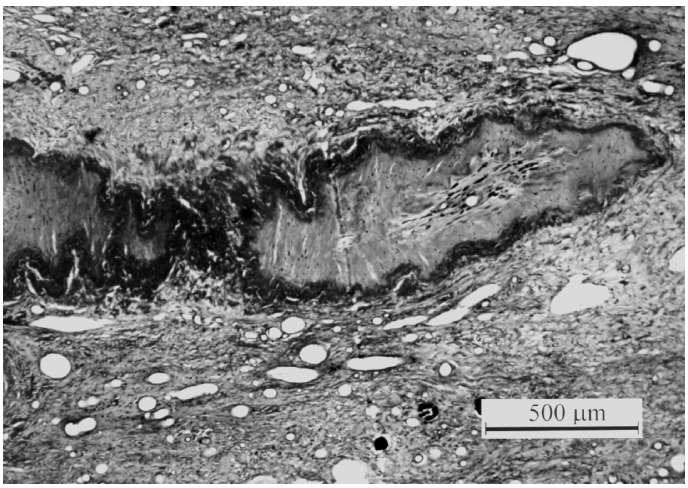

Figure 4: Recanalization of an artery within the main cryoinjured region stained with Masson's trichrome stain at 2 months post-cryosurgery. The artery at the center shows replacement of the cryotreated lumen by scar tissue, and reestablishment of small vascular lumen at the center of that scar tissue. (Rabin et al., 1999)

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