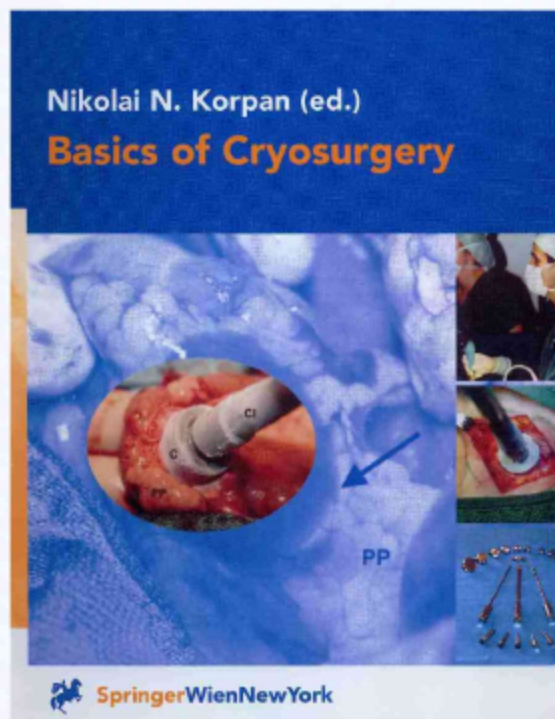




13.2 Minimally Invasive Breast Cryosurgery

Yoed Rabin with a contribution by Thomas B. Julian and Peter Olson

13.2.1 Why Minimally Invasive Breast Cryosurgery?

The treatment of carcinoma of the breast has followed a fascinating evolution through the centuries. In the seventeenth century treatment consisted of a barbaric amputation and hot iron cauterization technique, without the aid of anesthesia. Both surgical technology and the understanding of the biology of breast cancer developed slowly until the twentieth century. Breast cancer treatment was one of anecdotalism applied to

small numbers of patients without reproducible results.

During the twentieth century the treatment of breast cancer was dominated by the Halstedian principles of en bloc resection which mandated a radical mastectomy in order to control breast cancer. Halsted's belief that breast cancer spread systemically via contiguous involvement of muscle, fascia, and lymphatics was a strong one. Challenges to the Halstedian concept began to emerge and grow in the mid and late 1900's. These new concepts of local breast cancer control combined with a better understanding of the biological nature of the disease were incorporated into clinical trials. A landmark clinical trial from the NSABP B-06 provided data to support and propel breast conserving therapy forward (Fisher et al. 1995).

Currently, the utilization of breast conserving therapy has increased in the United States (Riley et al. 1999). As the information gained from a better understanding of the biological mechanism driving tumor cells is directed into new therapies which affect the cellular and subcellular level, breast conserving surgical procedures will take on a more significant role. Breast conserving therapy depends largely upon early detection, preoperative diagnosis and planning. Currently the best method for early detection is screening mammography (Tabar et al. 1985; Hendrick et al. 1997). Due to enhanced mammographic screening, it is estimated that by 2010 A.D. 50% of all new breast cancers discovered will be less than 10 mm in diameter (Cady et al. 1996), which represents 90,000 patients in the United States. Standard surgical treatments would require an open segmental resection, an operating room, anesthesia, and substantial cost.

The future, however, is bringing with it new tools which will undoubtedly increase the early detection of breast cancer and increase surgical intervention. On the horizon high resolution MRI is advancing rapidly in lesion detection, with sensitivities of nearly 100% (Harms et al. 1993; Orel et al. 1994). This technology may increase detection in the high risk population or of occult carcinomas. Digital mammography has the potential to improve detection and characterization of breast lesions.

Nuclear medicine scans utilizing scintimammography or PET are in both clinical and experimental use. Imaging may be achievable to a resolution of 1–2 mm. Each of these technologies has the ability to increase the detection of breast cancer and increase surgical removal of the disease at an earlier time.

The role of neoadjuvant chemotherapy is taking on an increasingly important role in breast cancer treatment. Results published from NSABP B-18 indicate a decrease in tumor size in 80% of patients with a modest conversion from mastectomy to lumpectomy (Fisher et al. 1998). Thus the use of preoperative chemotherapy can and has increased the role of breast conserving therapy. The effect of improving detection technology and preoperative therapies will ultimately increase segmental resections. The increase in surgical requirements will proportionally increase care costs.

Alternative methods of tumor removal or ablation for small malignancies are needed to complete the biological assault on breast cancer. A side benefit may be the lowering of treatment costs as well. Several technologies which have the ability to either remove tumor percutaneously or ablate tumor *in situ* percutaneously, are undergoing evaluation. Percutaneous tumor removal utilizing a vacuum-assisted stereotactic 11-gauge breast biopsy needle shows early promise (Lieberman et al. 1999), but questions need to be answered concerning margin status.

Radiofrequency ablation has proved to be feasible in breast tissue and breast cancer (Bohm et al. 2000; Jeffrey et al. 1999). Interstitial laser coagulation in breast cancer is undergoing study with promising results (Harms 2000). Both techniques require intravenous or general anesthesia.

An additional alternative means is cryosurgery. Cryosurgery has been used successfully for more than three decades to treat benign and malignant neoplasms (Rand et al. 1968; Zacarian 1977; Ablin 1980; Orpwood 1981). 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 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 anesthetic 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 (Rand et al. 1968; Ablin 1980; Ablin 1995; Suzuki 1995; Tonoka 1995).

Cryosurgery is still at a stage where art overshadows science. Most cryotherapy devices are bulky and somewhat complex to handle outside of a standard operating room. The ideal device would be small and compact. It should interface with the stereotactic biopsy table and ultrasound

devices. Debate continues as to numbers and duration of freeze-thaw cycles needed to maximize the probability of cell destruction. Little information is known about the survival of the cell at the freeze front and the ability to regenerate. This leads to questions about the optimum tumor size and dimensions of the "ice ball". Investigations into these questions and technology developments are ongoing (Rabin et al. 1997; Gage and Baust 1998; Rabin et al. 1999a).

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

The use of minimally invasive percutaneous cryosurgery is a technology which might provide a substitute for an open surgical segmental resection (lumpectomy). This technique may decrease the disadvantages of lumpectomy while enhancing the cosmetic effect of breast conserving therapy and promote biologic and immunologic response. Further investigations will need to be undertaken in these areas.

13.2.2 Technological Background

James Arnott (1845) was probably the first to report on the destructive effect of freezing in the treatment of cancer. Since Arnott's first report, numerous cryodevices and techniques have been suggested. These have included pre-cooled metal blocks, dry ice applications, spray/pour freezing with compressed or liquefied gases (Kollner and Duczek 1974), refrigeration systems (Timmerhausen 1989), thermoelectric methods and cryogenic heat pipes (Hamilton and Hu 1993), cryoneedles (Weshahy 1993), pre-cooled needles (Gao et al. 1986), heat conducting needles (Stumpf and Andrea 1974), Joule-Thompson effect based cryoprobes (Rzasa and Wallach 1983; Bald 1984; Varney 1992; Goddard 1993; Homasson et al. 1994; Maytal 1997), boiling effect based cryoprobes (Cooper and Lee 1961; Lee 1967; Zimmer 1975; Fowle 1994; Rabin et al. 1996a; 1996b), and super-cooled liquified gases (Rubinsky et al. 1994; Chang et al. 1994; Baust et al. 1996).

As a consequence of the high cooling power usually required for cryosurgery, and especially of

internal organs, the boiling effect has been found to be the preferable cooling technique by most cryosurgeons since the 1930's, when technological developments enabled commercialization of liquefied gases. Some of the technical difficulties associated with Joule-Thomson cooling for cryosurgery have been solved in the past few years and devices based on this cooling technique are now competing with those based on boiling cooling (Kaplan et al. 1995; Hewitt et al. 1997; Rewcastle et al. 1999).

Some of the cryoprobes for invasive procedures combine a heating mechanism near the tip of the cryoprobe, in order to enhance the control of the cryoprobe temperature and, hence, to gain a better control over the ice ball formation (Van Gerven 1980; Merry and Smidebush 1990; Rabin 1996a). It has recently been suggested to combine temperature-controlled heaters (also termed Cryoheaters) within the cryotreated region, that are operated independently of the cryoprobes, in order to control the frozen region formation more precisely (Rabin et al. 1999b).

Cryosurgery can be monitored by ultrasound (Onik et al. 1985; Onik et al. 1988; Onik et al. 1991; Staren et al. 1997), CT (Isoda 1989; Quigley et al. 1992; Sandison et al. 1998), and MRI (Rubinsky et al. 1993; Gilbert et al. 1993; Hong et al. 1994), where ultrasound is most commonly used today. In addition, verification of temperatures and freezing front location can be achieved using point sensors such as impedance electrodes (Le Pivert et al. 1977; Novak and Craig 1984; Gage et al. 1985) and thermocouples (Rivoire et al. 1996; Abramovits et al. 1996). However, due to difficulties in sensor localization and uncertainty in measurements, point sensors are rarely a choice of practice in routine cryosurgery (Rabin 1998a).

Following is an up-to-date report on our research group efforts in development of a cryosurgical device for minimally invasive cryosurgery, experimental cryosurgery in sheep breast model, and long-term follow-up post cryosurgery.

13.2.3 A Compact Device for Breast Cryosurgery

For practical reasons of cost and simplicity, we chose liquid nitrogen boiling as the cooling technique for the development of a compact device and miniature cryoprobe for breast cryosurgery (Rabin et al. 1997). One of the key questions for thermal design is "What are the cooling capabilities required of such a system?" The ratio of the energy required to freeze a unit volume of tissue to

the energy that can be absorbed by a unit volume of liquid nitrogen in boiling can be approximated by:

$$\eta = \frac{\int_{T_0}^{T_F} C_U dT + L_F + \int_{T_F}^{T_C} C_F dT}{L_{LN}} \quad (1)$$

where η is the energy ratio, T_0 is the initial temperature of the tissue (normal body temperature), T_F is the freezing temperature of the tissue, T_C is some minimal cryogenic temperature, T_{LN} is the liquid nitrogen boiling temperature, C_U is the volumetric specific heat of the unfrozen tissue, C_F is the volumetric specific heat of the frozen tissue, L_F is the latent heat of tissue freezing, and L_{LN} is the latent heat of liquid nitrogen boiling. Equation (1) is valid for cases of low blood flow rate, where the heat convection by blood is not significant. Nevertheless, Rabin and Shitzer (1998c) have shown theoretically that the frozen region size should not be affected significantly even in cases of high blood flow, as long as the cryotreatment is not being performed in the presence of a major blood vessel.

Applying the parameters listed in Table 13.2.1, Eq. (1) yields an energy ratio, η , in the range of 2 to 4. This means that, theoretically, freezing 1 ml of tissue requires only 2 to 4 ml of liquid nitrogen. It follows that, freezing a 1 cm spherical ice ball requires 1 ml to 2 ml of liquid nitrogen, freezing a 2 cm ice ball requires 8 ml to 16 ml of liquid nitrogen, and freezing a 5 cm ice ball requires 125 ml to 250 ml. Clearly, high thermal efficiency is a key parameter for the design of a compact cryodevice. Note that the parameter of thermal efficiency is typically overlooked in the design of cryodevices. Furthermore, liquid nitrogen-based cryodevices for internal organs are typically characterized by extremely high coolant consumption and, therefore, by low thermal efficiency.

A new device for minimally invasive cryosurgery, characterized by high thermal efficiency, has been presented by Rabin et al. (1997), and is describe in brief herein. The new minimally invasive cryoprobe comprises a U-shaped heat exchanger, a sharp pointed tip, and a thermal insulation jacket; one configuration of the cryoprobe is shown in Fig. 13.2.1.

The cooling process in the cryoprobe shown in Fig. 13.2.1 takes place as follows. Liquid nitrogen is forced from a small, hand held, container into the cryoprobe through feeding tube 9. The liquid nitrogen flows along the cryoneedle towards the

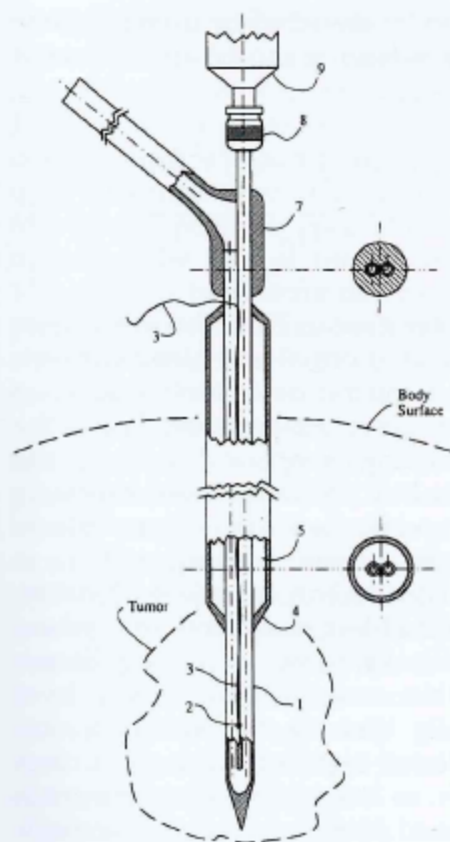


Fig. 13.2.1. Schematic illustration of a cross-section of a minimally invasive cryoprobe for breast cryosurgery (Rabin et al. 1997)

tip and then back, towards the outlet tube. Thermal insulation jacket 4 (achieved by vacuum in gap 5) prevents freezing of surrounding tissues. The only significant heat transfer occurs where the cryoneedle is in direct contact with the tissue, designated as the active surface, 1. Downstream heat convection driven by boiling effect takes place along the cryoprobe active surface, and causes freezing of the tissue. To achieve high thermal efficiency, the liquid nitrogen flow rate has to be controlled to ensure that no nitrogen droplets will escape to the surroundings through the venting tube 7.

Using an 18.5-gauge hypodermic needle, as construction material for the cryoneedle heat exchanger, and liquid nitrogen pressure of 30 psi, the typical variation of temperature at the cryoprobe active surface is: 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 generates an average frozen region diameter of 22.3 mm within 5 min of operation in sheep breast tissue ($n = 21$). This cryosurgical device has a high thermal efficiency

(43%). More detail with regard to the cryodevice setup and validation testing is presented by Rabin et al. (1997).

13.2.4 Cryoprobe Localization

Cryoprobe localization for internal applications can be performed using an imaging technique such as ultrasound or MRI, where the cryoprobe tip is simply maneuvered towards the center of the target area to be cryotreated. For example, the cryoprobe can be localized in a similar manner as in the well established process of needle localization technique, as a pre-process for conventional breast lumpectomy.

The flexibility of breast tissue increases the degree of complication in localization of a minimally invasive cryoprobe. In order to overcome this difficulty, one can use the highly accurate and well established technique of stereotactic localization for biopsy needles. In this procedure, the breast is imaged by two low-intensity X-ray images, at an angle of 15° from one another. The data is fed to a computer which generates a 3D image of the breast. Once the operator identifies a specific spot on these two images, the computerized device calculates the coordinates of the specific spot in space and the biopsy needle is automatically driven accordingly. Figure 13.2.2 shows 3 minimally invasive cryoprobes having thermal insulation jackets in a diameter of 3 mm and active surface length of 22 mm (upper, black), 32 mm

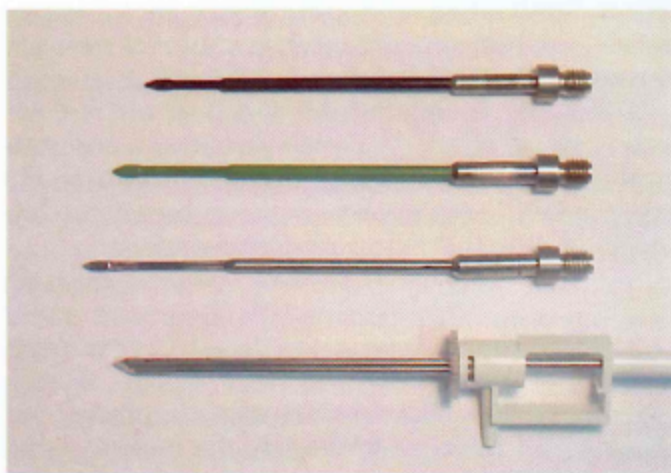


Fig. 13.2.2. Minimally invasive cryoprobes having a thermal insulation diameter of 3 mm, cryoneedle tube diameter of 1.5 mm, and active surface length of 22 mm (top, black), 32 mm (second from above, green), and 42 mm (third from above, stainless steel). Below, a biopsy needle from Biopsys Medical, Inc. (Mammotome Multi-Probe), having an oval cross-section of 3 x 4 mm

(second from above, green), and 42 mm (third from above, stainless steel). For comparison, Fig. 13.2.2 also shows a standard biopsy needle of Biopsy Medical, Inc. (below). One can see that the new cryoprobes can be easily designed to be compatible with the biopsy needle. It follows that stereotactic cryoprobe localization can be applied to breast cryosurgery.

13.2.5 Experimental Cryosurgery in a Sheep Breast Model

The animal model for this study is a recently pregnant sheep, 8 to 12 weeks post-lambing, and at least 4 weeks post-lactation. All animals in this study were about 5 years old, after 5 deliveries (once a year), having body weight in the range of 40 kg to 80 kg. Under these conditions the sheep breast is similar in size to the human breast. The importance of similarity arises from: (i) the need of large enough volume of untreated tissue surrounding the cryoinjured site, as the surrounding tissue is expected to take an important role in the recovery and regeneration process; and, (ii) the need of large size to make imaging practical.

All cryotreatments were performed on healthy breast tissue and not on a tumor model, the underlying assumption being that the recovery and regeneration processes following cryoinjury are not dependent on the pre-existence of a breast tumor. Based on the reported results of 100% tumor kill with cryosurgery of breast cancer in small animals (Staren et al. 1997), it is further assumed that cryodestruction of breast tumors is feasible. 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 breast. Therefore, the cryotreatment of healthy breast tissue is a choice of practice.

Cryoprocures were performed in areas of dense breast tissue fibers and as far as possible from the dilated breast ducts. Identification of the different areas of the breast and monitoring of the ice ball formation were performed using a Doppler ultrasound (7 MHz linear array transducer). Figure 13.2.3 shows an ultrasound monitored cryoprocure, where the ultrasound transducer is handheld from above, perpendicular to the cryoneedle center line, and above the middle of its active surface, with the animal lying on its back. The ultrasound images were videotaped for further analysis and measurements. In Fig. 13.2.3, the cryoprobe is inserted horizontally into the right



Fig. 13.2.3. Experimental cryosurgery in a sheep breast model. The animal is lying on its back. The ultrasound transducer is handheld from above. The cryoprobe is inserted horizontally and localized in an area of dense breast fibers

breast. Figure 13.2.4 shows a closer view of the point of penetration into the breast, where a small cut (3 to 5 mm in length) in the skin was prepared prior to cryoprobe insertion and was closed with one suture at the end of the operation.

Figure 13.2.5 shows ultrasound images of the cryotreated site as the cryoprocure progresses. Figure 13.2.5(a) shows the imaged cryoneedle prior to freezing, circled with a dashed line. Figure 13.2.5(a) also shows the shadow of the needle

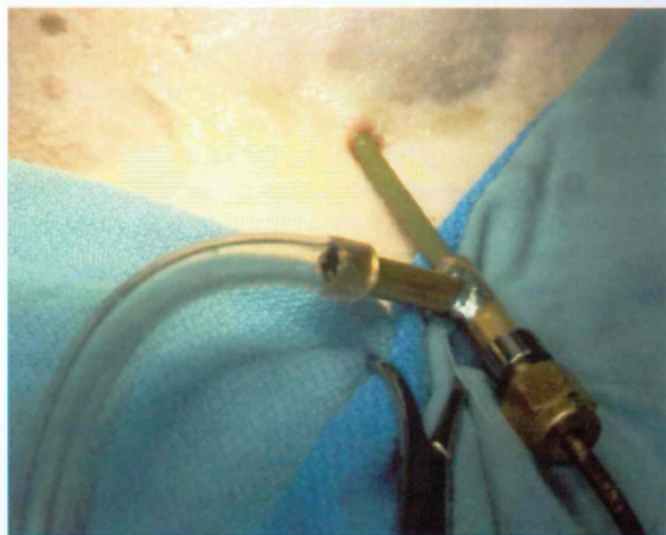


Fig. 13.2.4. A small skin cut of 3 to 5 mm is made to allow smooth cryoprobe penetration. The cryoprobe does not enhance the injury at the point of penetration. At the end of the cryoprocure, one skin suture is applied to close the penetration point

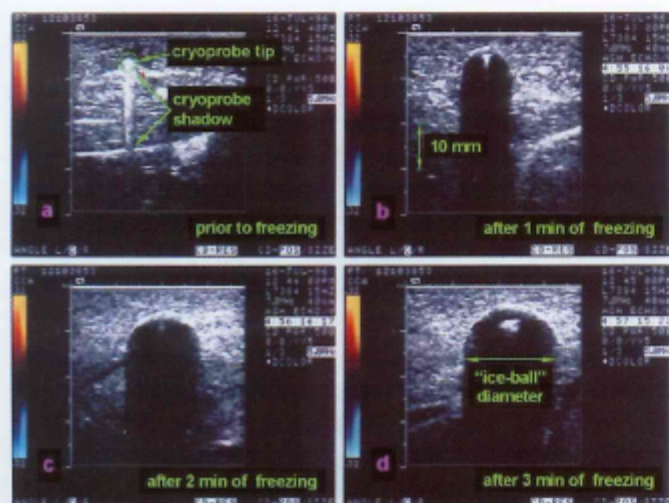


Fig. 13.2.5. Ultrasound imaged "ice ball" formation in a sheep breast model using 7 MHz linear array transducer. The frozen region appears as a dark area in the ultrasound image. The cryoprobe is placed perpendicular to the ultrasound transducer (to the monitor), the ultrasound transducer is applied from above and, therefore, the cryoprobe shadow is projected downwards, as illustrated in a. b–d show ultrasound images after 1, 2, and 3 min from the beginning of freezing. A measurement of the ice ball diameter is illustrated in d, which appears to be about 2 cm after 3 min of cryo-operation in this particular procedure

in ultrasound imaging. Shadowing is an inherent property of ultrasound imaging in the presence of solid objects. As the cryoprocure progresses, a frozen region forms around the cryoprobe, appearing as a dark area, accompanied by a dark shadow projected downwards (the frozen tissue is solid). Figures 13.2.5(b), 13.2.5(c), and 13.2.5(d), show the ultrasound image after 1, 2, and 3 min from the beginning of cryo-operation. A length bar is illustrated in Fig. 13.2.5(b), while the frozen region diameter is illustrated in Fig. 13.2.5(d). It follows that, in this particular cryoprocure, a frozen region diameter of about 10 mm, 16 mm, and 20 mm, was measured after 1 min, 2 min, and 3 min from the beginning of cryo-operation. This technique of frozen region diameter was repeated in 28 cases to generate a data base of the frozen region development in breast tissue under various conditions. The ultrasound imaged ice ball was compared with histology findings. For this comparison a staining technique was developed to assess the extent of cryoinjury, as discussed below.

13.2.6 Tissue Fixation and TTC Application as a Cryoinjury Indicator

The extent of cryoinjury was evaluated using the vital stain 2,3,5-triphenyltetrazolium chloride

(TTC), an oxidation – reduction indicator that has been used effectively for histochemical analysis of infarct volume in ischemically injured tissues (Rabin et al. 1998b; 1999a). TTC, as a water soluble salt, is not a dye but is reduced by certain mitochondrial respiratory enzymes in normal tissue to a deep red, fat soluble, light sensitive compound (formazan) that turns normal tissue brick red and thereby delineates abnormal areas. TTC has been used extensively to stain tissues from humans and experimental animals and has been shown to reflect accurately the extent of irreversible ischemic damage.

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* (Rabin et al. 1998b). In brief, all the major veins leading from the breasts were exposed and ligated (Fig. 13.2.6). The two major arteries leading to the breasts were exposed, cannulated, and connected to a controlled flow rate pump via a T connector. The breasts were perfused with 250 ml of TTC 2% in phosphate-buffered saline, at a rate of 18 ml/min, followed by 100 ml of 37.2% formaldehyde at a rate of 18 ml/min. The TTC and the animal's breasts

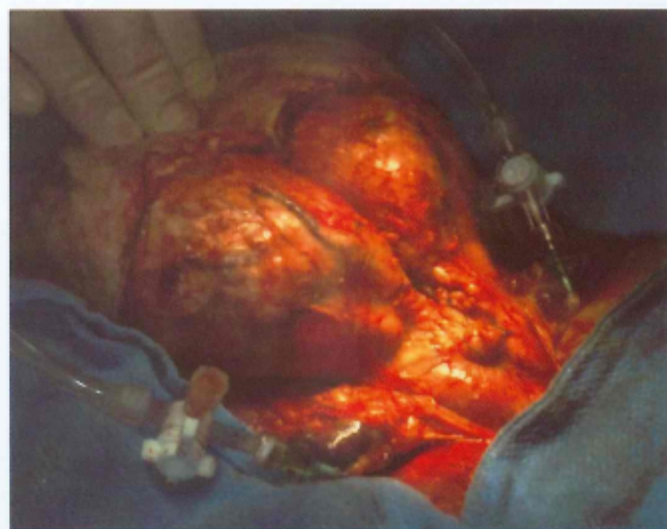


Fig. 13.2.6. Tissue fixation and staining with the vital stain 2,3,5-triphenyltetrazolium chloride (TTC): All the major veins leading from the breasts were exposed and ligated. The two major arteries leading to the breasts were exposed, cannulated and connected to a Harvard syringe pump via a T connector (the catheters are shown at both sides of the breasts). The breasts were perfused with 250 ml of 2,3,5-triphenyltetrazolium chloride (TTC) 2% in phosphate-buffered saline, at a rate of 18 ml/min, followed by 150 ml of 10% natural buffered formaldehyde at a rate of 18 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

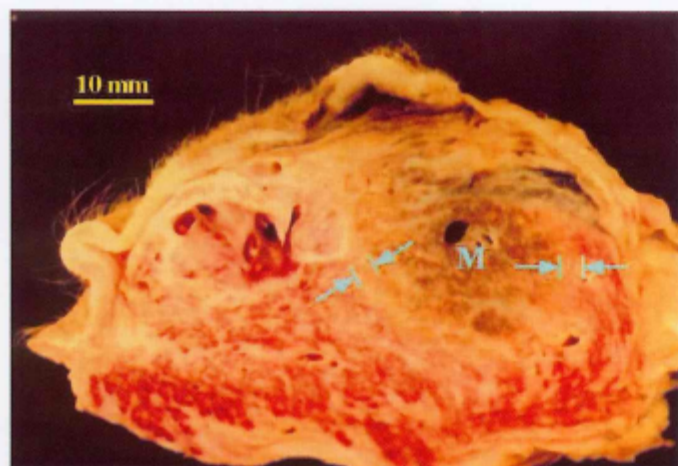


Fig. 13.2.7. Cross-section of a fixed breast specimen stained with TTC immediately after thawing. The main cryoinjured region is designated by *M*. The partly injured region, designated as the transition zone between the main cryoinjured region and the surrounding healthy tissues, is pointed out by blue arrows

were maintained at 37°C throughout the procedure for optimal histochemical enzyme reduction in the tissue using a temperature controlled thermal blanket. Skin temperature measurements were taken to verify temperature control, using 36 gauge hypodermic thermocouples. The breasts were excised immediately after and immersed in 10% neutral buffered formaldehyde solution for up to 24 h. The specimens were then bisected along the cryoneedle track, the color of the cut surface was observed and the cryoinjured (discolored) region was measured (Fig. 13.2.7).

Representative blocks of tissue from the cryotreated region were submitted for standard histological examination by light microscopy using hematoxylin & eosin stain (H&E), and Masson's trichrome stain. Representative histology cross-sections are presented.

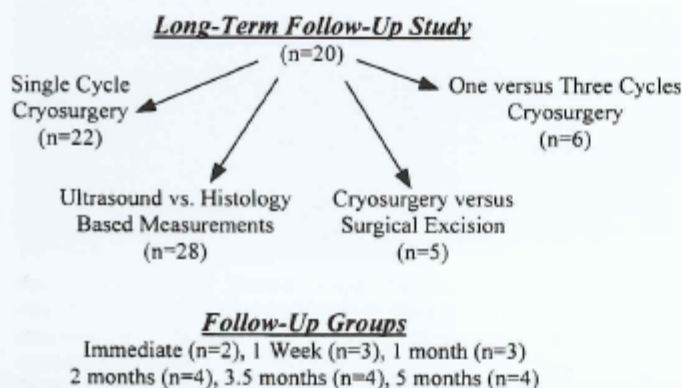


Fig. 13.2.8. Summary of animal work in the long-term follow-up study

13.2.7 Long-Term Follow-Up Post-Cryosurgery

The long-term follow-up study is described in detail by Rabin et al. (1999a). In brief, with reference to Fig. 13.2.8, a total of 20 animals were studied in six follow-up groups. Follow-up groups of immediate (n = 2), 1 week (n = 3), 1 month (n = 3), 2 months (n = 4), 3.5 months (n = 4), and 5 months (n = 4), were selected. The immediate follow-up experiments were performed to test the cryoprobes, to provide a baseline for the long-term follow-up, and to improve the application of the TTC perfusion and *in situ* fixation as described above.

A single cryoprocure on one breast was compared with a three cycle cryoprocure in the other breast in six animals of the 1- to 5-month follow-up groups. Five animals were used to compare the scar tissue developed as a result of a surgical excision with that developed after a single cycle cryoprocure, for the same 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. One skin suture was applied to close the incision created for the cryoprobe, while about 4 sutures were applied to the surgical excision.

MRI and mammography were obtained about every 4 weeks in an effort to identify the sites of cryotreatment. The MRI was performed on animals from the 5-month follow-up group, under general anesthesia, with the animals lying on their backs, in a Siemens® MRI unit with 1.5 Tesla intensity. 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. In some cases, the mammography was performed *in vivo* under general anesthesia, with the animals lying on their sides. In other cases, the mammography was performed on the breast specimens immediately after harvesting.

Figure 13.2.7 shows a cross-section of a fixed breast specimen stained with TTC from the immediate follow-up group. The main cryoinjured region is designated by the letter *M*. The partly injured region, designated as the transition zone between the main cryoinjured region and the surrounding healthy tissue, is pointed out by blue arrows. High power magnification of the main cryoinjured region is shown in the top right field of Fig. 13.2.11, and of the transition zone at the top left of Fig. 13.2.12. For comparison, the normal glands of the breast stained with H&E are shown at the top left of Fig. 13.2.11 (control).

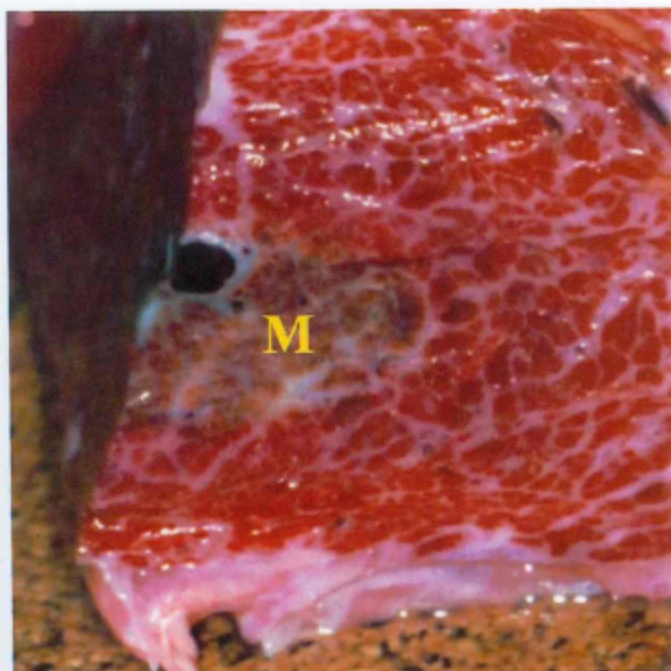


Fig. 13.2.9. Cross-section of a fixed breast specimen stained with TTC 7 days post-cryosurgery. The main cryoinjured region is designated by M

Figure 13.2.9 shows a cross-section of a fixed breast specimen stained with TTC 7 days post-cryosurgery. The main cryoinjured region is designated by the letter M. Necrosis with complete loss of cellular detail is demonstrated at 1 week post-cryosurgery, bottom left field of Fig. 13.2.11.

Figure 13.2.10 shows a cross-section of a fixed breast specimen stained with TTC at 1 month post-cryosurgery. The main cryoinjured region is designated by the letter M. The transition zone is designated by the letter T and pointed out by the yellow arrows. The bottom right field of Fig. 13.2.12 shows regenerating duct in loose stroma, at 1 month post-cryosurgery (stained with Masson's trichrome stain). The Masson's trichrome stain is used to distinguish fibers within the scar. The trichrome dye stains collagen green, cytoplasm red, and nuclei black, in contrast to the standard H&E stain, which gives a pink color to both collagen fibers and cytoplasm of fibroblasts.

The cryoinjured site is very difficult to observe macroscopically at 5 months post-cryosurgery. However, microscopic findings show capillaries, fibroblasts, and bundles of collagen at 5 months post-cryosurgery (bottom right of Fig. 13.2.11), which indicate regeneration and recovery in progress.

13.2.8 Up-to-Date Summary

A minimally invasive cryosurgical device has been developed. The new cryosurgical device generates

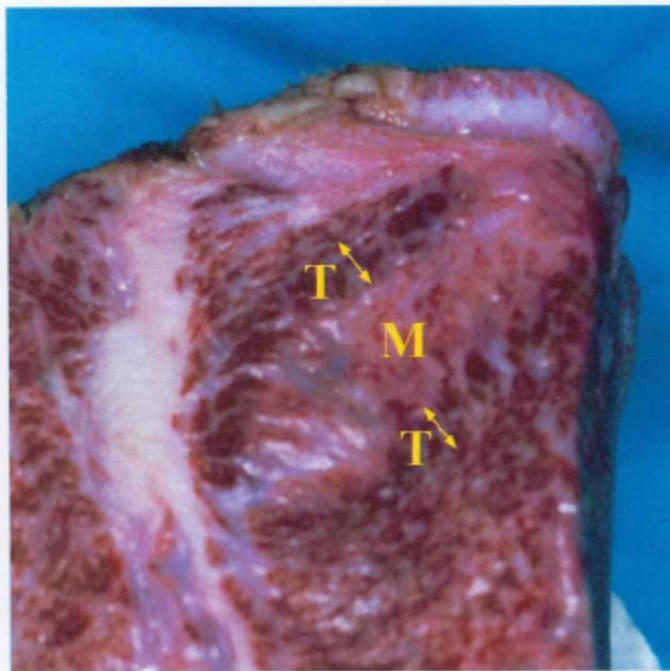


Fig. 13.2.10. Cross-section of a fixed breast specimen stained with TTC 1 month post cryosurgery. The main cryoinjured region is designated by M. The transition zone is designated by T and pointed out by the yellow arrows

sufficient cooling power for breast cryosurgery with a relatively low coolant consumption, where 43% thermal efficiency has been demonstrated in gelatin. The new cryodevice can generate ice ball

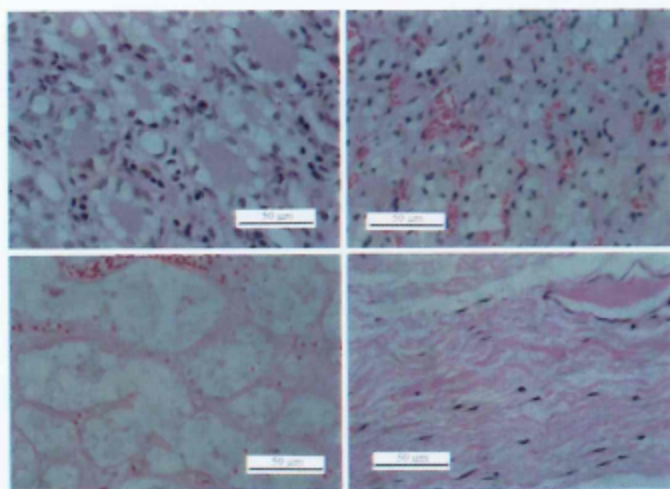


Fig. 13.2.11. Main cryoinjured region stained with H&E. Top left: Control tissue, normal glands of the breast. Top right: loss of cytoplasmic and nuclear detail immediately post-cryosurgery. Bottom left: necrosis with complete loss of cellular detail at 1 week post-cryosurgery. Bottom right: capillaries, fibroblasts, and bundle of collagen at 5 months post-cryosurgery (Fig. 13.2.11 is reproduced from Rabin et al. 1999a)

diameter of 20 mm within 3 to 5 min in breast tissue.

Experimental results indicate that the growth of the frozen region in sheep breast tissue is: (i) slower in larger breasts, (ii) slower in areas of large ducts and high fluid content, and (iii) faster in areas of fibrous breast tissue. When compared with liver cryosurgery, the frozen region growth in the sheep liver is 3 to 4.5 times faster than in the sheep breasts.

Ultrasound imaging of the ice ball formation leads to an underestimation of the cryoinjured region in the range of 2 to 5 mm. The cryoinjured region dimensions include a transition zone of partly damaged lobules having a typical thickness of 2 to 5 mm. A conservative application of the cryosurgical device developed for the current

study in breast tissue suggests 5 mm safety margins in an ultrasound monitored cryoprocure. 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 cryoprocure. The reduction in cryoinjured region size over time is probably the result of contraction of the scar tissue within the area of injury as the scar develops, the post-injury healing process of the tissue, and the natural reduction of the sheep breast over time post-lambing. The cryotreatment site in a sheep breast model cannot be identified up to 5 months post-cryosurgery by means of ultrasound,

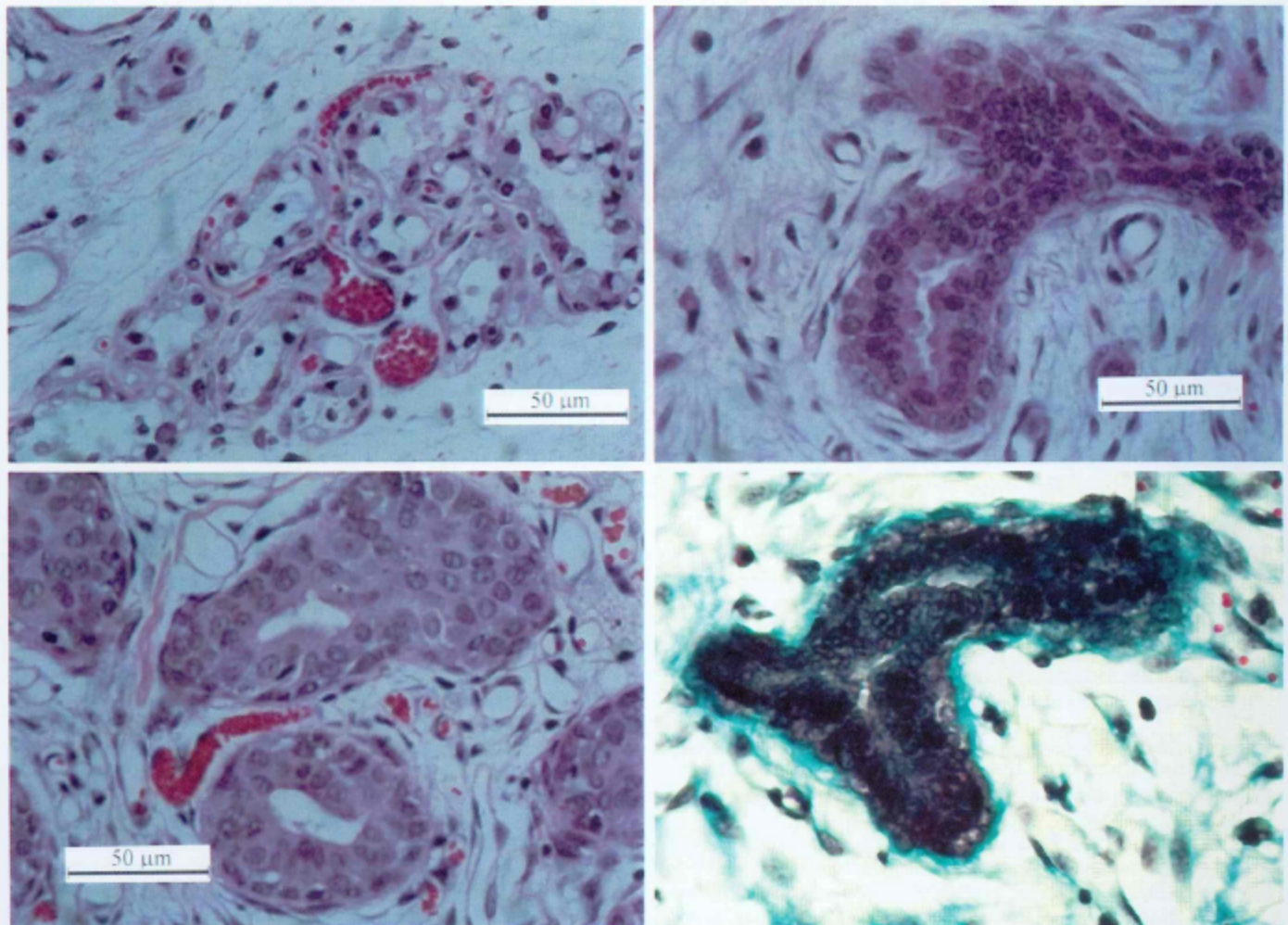


Fig. 13.2.12. Transition zone between the main cryoinjured region and the surrounding healthy tissues. Top left: glands with interstitial edema and vascular congestion with red blood cells immediately post-cryosurgery (H&E). Top right: regenerating duct in loose stroma at 1 month post-cryosurgery (H&E). Bottom left: regenerating duct, no secretory glands at 5 months post-cryosurgery (H&E). Bottom right: regenerating duct in loose stroma at 1 month post-cryosurgery (Masson's trichrome stain). The Masson's trichrome stain is used to distinguish fibers within the scar. The trichrome dye stains collagen green, cytoplasm red, and nuclei black, in contrast to the standard H&E stain, which gives a pink color to both collagen fibers and cytoplasm of fibroblasts. (Fig. 12.2.12 is reproduced from Rabin et al. 1999a)

mammography, or MRI. Using these standard imaging techniques, it is highly likely that the scar tissue will not be misinterpreted as a potential tumor in the long term.

The cryoprocure 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. There is no gross or microscopic difference between lesions that have been subjected to one versus three freeze/thaw cycles. Either way, there is a main cryoinjured region that has uniform destruction of epithelium and healing scar formation, and a transition zone of damaged lobules without acining, which are surrounded by healthy tissue.

In terms of recovery and regeneration, surgical excision appears to have an advantage over cryosurgery, which is a more rapid healing process. The cryosurgical wound will catch up in establishing a fibrous scar in time, perhaps longer than one year. As a surgical procedure, however, cryosurgery has the advantages of substantial decrease in the risk of morbidity, simplicity of the procedure, minimal bleeding, anesthetic effect of low temperatures, low cost, minimal scarring, and possible stimulation of the body's immune system.

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