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TISSUE SAMPLING METHODS AND THE EFFECTS OF
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TECHNIQUES IN THE DETECTION AND TREATMENT OF BREAST CANCER: TISSUE SAMPLING METHODS AND THE EFFECTS OF TISSUE DESTRUCTION DIRECT ELECTRIC CURRENT

By

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ABSTRACT

TECHNIQUES IN DETECTION AND TREATMENT OF BREAST CANCER:
TISSUE SAMPLING METHODS AND THE EFFECTS OF
TISSUE DESTRUCTION BY DIRECT ELECTRIC CURRENT

XiaoTan Qiao

This study had two objectives. The first objective was to compare the fine needle aspiration biopsy (FNA) and Rotex needle biopsy techniques (RNB). The quantity and the quality of cells and the tissue fragments of specimens obtained with FNA and RNB were analyzed statistically. This study demonstrated that the RNB produces a greater quantity and a higher quality sample for interpretation than does FNA. Correlations between cell numbers and fragment numbers varied with the different tissue types. The second objective was to observe the tissue changes during and after the application of direct electrical current (DC). The tissue damage included necrosis. These experiments indicated that 10 - 20 volts is the optimal voltage needed to produce the desired degree of tissue damage if this technique were to be used in treatment of cancer or other unwanted tissues.

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INTRODUCTION

Breast cancer is the most common malignant disease in women of the western world (Factors, 1991), and is the leading cause of death from cancer in female between 40 to 45 years of age (Stockdale, 1990). Approximately 1 in every 10 American women will develop breast cancer (Gold, Bassett and Kimme-Smith, 1987), and only about one half of this group can be successfully treated (Factors, 1991). Thus, efforts are underway to find more effective methods for prevention, detection, and treatment of breast cancer (Oakar, 1992; Harness, 1988; Smalley and Oldham, 1988).

One of the more recently accepted approaches to the treatment of breast disease incorporates stereotactic mammographic localization of lesions. This latter procedure particularly offers the advantage of an early diagnosis and local treatment through its incorporation of mammography and fine needle aspirations (FNA) and Rotex needle biopsy (RNB). Another potentially useful approach which uses these needle techniques, incorporated the application of local direct electric current (DC) therapy to destroy cancerous tissue (Bolmgren, Jacobson and Nordenström, 1977; Nordenström,

1983).

The concept of mammography was developed in 1913 by Salomon, a pathologist, to define more precisely the morphologic characterizations of tumors in the breast (quoted in Dodd, 1989). In 1927, mammography was performed by Kleinschmidt in vivo (quoted in Pennes and Adler, 1988). Three years later, 119 cases which included 58 malignant tumors were reported by Warren (Warren, 1930; Gold, Bassett, and Kimme-Smith, 1987). Early mammography images were of poor quality, but by 1960 mammographic techniques were much improved (Egan, 1960). In 1964 Payr was the first one to perform mammograms on clinical patients (Payr, 1964). Thus mammography became an important technique for detecting breast disease and a unique method for detecting nonpalpable breast masses (Weiss and Wayrynen, 1976; Gold, Bassett and Kimme-Smith, 1987). In the past decade, mammographic techniques have made possible the early detection of nonpalpable, early breast cancers and have resulted in a reduction in the mortality and morbidity from breast cancer (Holleb, 1992).

Although a presumptive diagnosis of breast cancer can be made from a patient's history, and a physical examination, or from radiological studies, one of the major problems with the mammographic diagnosis of breast cancer is its variable and sometimes subtle radiographic appearance. Therefore, the biopsy of breast abnormalities is necessary and pathological examination still essential for the confirmation of a

diagnosis (Souba and Bland, 1991). The use of specialized techniques, such as magnification and conedown radiography, as well as FNA of nonpalpable lesions, frequently allows the preoperative diagnosis of malignancy to be of a high degree of certainty. In addition to the use of FNA, Nordenström reported using a Rotex needle to biopsy mammary tumors in 1975. Two years later, Bolmgren and his colleagues reported the use of a stereotactic instrument for assisting FNA and RNB. In 1989, Azavedo and his colleagues reported using stereotactic instruments for fine needle aspiration biopsy (SFNA) and Rotex needle biopsy (SRNB) in 2594 nonpalpable breast lesions (Azavedo, Svane and Auer, 1989) with the biopsy material used for morphological evaluation, and for assessment of tumors' biological properties, such as tumor nuclear DNA. This group also reported that in 77.3% of patients, surgery was avoided because SFNA and SRNB technique had been performed (Azavedo, 1989).

In 1983, Nordenström published his work on the use of direct current electrodes, positioned by the stereotactic instrument, to treat breast cancer locally, and reported remarkably successful results. In 1989, Quan used DC on 62 patients with malignant tumors (including 13 with breast cancers), and Xin reported 127 patients with malignant tumors (including 17 with breast cancers), treated with DC with an effectiveness of 93.5% and 77.1% respectively. DC local treatment was proposed as a new method for the treatment of

malignant tumors.

Potchen, Sierra and Mackenzie introduced the use of stereotactic Rotex needle biopsy technique specifically on breast tissue into the United States in 1990 (Potchen, et al, 1991). Stereotactic localization for fine needle aspiration and the "Biopsygun" biopsy techniques in the detection of breast disease has gained much attention in recent times. Stereotactic fine needle aspiration and Rotex needle biopsy techniques with direct current (DC) treatment have the potential not only for prevention and detection, but also for the treatment of and research into breast cancer (Azavedo, 1989; Azavedo, Svane and Auer, 1989; Nordenström, 1983; Taubes, 1986).

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CHAPTER ONE

TISSUE SAMPLING: A COMPARISON OF THE WEIGHT AND THE CELLULAR NATURE OF THE MATERIAL OBTAINED BY FINE NEEDLE ASPIRATION AND ROTEX NEEDLE BIOPSY TECHNIQUES

BACKGROUND

The use of needle aspiration for the diagnosis of breast diseases has had more than 60 years of tortuous history. As early as 1926, Martin and Ellis used an 18 gauge needle with a syringe for sampling tumors from patients with cancer and related diseases. In 1930, they published their data for 65 malignant tumors and this heralded the introduction of the needle aspiration biopsy technique to diagnostic medicine (Martin and Ellis, 1930; Schondorf and Schneider, 1978). In 1933, Stewart, a pathologist, published his experiences with cytopathologic diagnosis (Rosen et al, 1972). One year later, Martin and Ellis reported results from 1,400 needle aspiration biopsies. They emphasized that this method offered the advantages of shortened anesthesia time and the establishment of a preoperative diagnosis. As a result, the needle aspiration biopsy became a widely utilized diagnostic procedure (Martin and Ellis, 1934; Feldman and Covell, 1985).

About 13 years later, in 1947, needle aspiration was criticized by Ochsner and DeBakey when they found tumor implants had developed in three patients after needle aspiration biopsies. Their conclusion was that this procedure should not be used in operable patients (Ochsner and DeBakey, 1947; Feldman and Covell, 1985). Similar concerns about the needle aspiration biopsy were published by others, all of which resulted in restricted use of this procedure with a reduction in the number of the needle aspiration biopsy procedures in the United States at that time (Allbritten, Nealon and Gibbon, 1952; Schachter and Basta, 1973; Feldman and Covell, 1985). However, these conclusions were based on the 18 gauge needle aspiration biopsy procedure. Later, these worries were found not to be valid for biopsies when the 22 gauge needle was used in tissue sampling (Wilkinson, Franzini and Masood, 1991).

In the following years, investigators reviewed the results of over 22,000 biopsies (Godwin, 1956; Franzen and Zajicek, 1968; Zajicek, 1972; Feldman and Covell, 1985; Zajdela et al, 1975; Kline, 1979) and recommended the reintroduction of needle aspiration biopsies, but with the finer (22 gauge) needle replacing the larger needle for this procedure. Fine needle aspiration biopsy (FNA) is now used frequently in the United States to diagnose palpable lesions in the breast (Kern, 1979; Vetrani et al, 1992).

A major function of FNA biopsy is to separate benign

diseases from malignant tumors via pathological and biological studies (Strum, Phelps and McAtee, 1983; Azavedo, Svane and Auer, 1989). FNA combined with stereotactic mammographic localization has become the most reliable method for the diagnosis of nonpalpable breast cancer and other abnormalities in recent years (Kreula, 1990; Malberger et al, 1991; Vetrani et al, 1992; Mitnick et al, 1992).

The "Rotex" needle is not presently used as frequently as bevelled needles for obtaining samples for the interpretation of breast masses. Nordenström first used the Rotex needle to obtain cytologic material from mammary tumors in 1975 (Nordenström, 1975). Two years later, he performed biopsies by combining this instrument with stereotactic localization of mammographic lesions (Nordenström, 1977). This combined procedure was then adopted by the Karolinska Institute and Hospital of Sweden as a part of their standard biopsy procedure for the detection of nonpalpable breast cancers. This group, Azavedo and colleagues, reported their experiences with 2594 mammographically detected nonpalpable breast lesions. Stereotactic Rotex needle biopsy technique (SRNB) was considered by them to be a highly sensitive procedure for early diagnosis of breast cancer when used in diagnostic combination with mammography (Azavedo, Svane and Auer, 1989). In 1990, SRNB was first used with stereotactic fine needle aspiration biopsy in the United States for the detection of breast nonpalpable lesions by Potchen, Sierra and Mackenzie in

a joint program with the Swedish group (Potchen et al, 1991).

A successful needle biopsy sampling depends on three components being performed correctly: the biopsy procedure, the preparation of sample material, and the interpretation are all essential for the success of both FNA (Cohen et al, 1987) and for RNB. FNA biopsy performance has been thoroughly discussed by Koss, Fox and others (Koss, 1980; Fox, 1979; Feldman and Covell, 1985), as has the Rotex needle biopsy technique (Nordenström, 1977). Both needle types have now been used for many thousands of patients.

The fixation of the cellular material currently depends largely on the preference of the person carrying out the cytological examinations. Air drying fixation, followed by May-Grunwald Giemsa staining, is the most common method of preparation for cellular smears. Ninety-five percent ethyl alcohol is standard for use with the Papanicolaou stain and is also commonly used. Four percent formaldehyde with Feulgen stain is the method of choice for single cell cytophotometry, and 95% ice cold ethanol is the cellular fixation used for flow cytophotometry (Azavedo, et al, 1982; Verma and Kapila, 1989).

Other techniques that have been used for specimen fixation include the wet fixation technique in which there is immediate submersion of freshly obtained cells in fixative. Spray fixation technique incorporating ethanol, polyethylene glycol, and distilled water, is sometimes substituted as a

coating fixative for the smear fixation. This procedure results in better cellular morphology than air-dry fixation does (Sheehan and Hrapchak, 1980; Danos and Keebler, 1975).

Procedures which use May-Grunwald-Giemsa, Papanicolaou or Diff-Quik stain are commonly used for FNA or Rotex needle biopsies (Vilaplana and Ayala, 1975; Schondorf, 1978; Azavedo, Svane and Auer, 1989).

MATERIALS AND METHODS

ANIMAL EXPERIMENTS

SPECIMENS

Three-month-old female CBA/J mice were used as donors of connective and muscle tissues. Four-month-old female Sprague Dawley rats were used for the adipose tissue studies.

Subdermal tissues were obtained from the dorsal, abdominal and iliac crest skin of the animal to provide representative connective and muscle tissues; the abdominal omenta of rats were sampled to provide adipose tissues.

EQUIPMENT AND SOLUTIONS

A CONTROL II INRAD analytical balance was used to weigh the samples.

The biopsy needles used were: a) 22 gauge bevelled aspiration needles obtained from Becton Dickinson and Company,

these were 115 mm in length, b) Rotex needles (172 mm long) made up of a 0.8 mm thick cannula with an inner 15 mm long groove, and 0.55 mm thick; they were obtained from URSUS KONSULT AB, Sweden. A 10 cc syringe with syringe-control II supplied by INRAD was used. The instrument holder was supplied by URSUS KONSULT AB, Sweden.

The glass slides used were precleaned and had a frosted end for labelling.

Standard May-Grunwald Giemsa stain solutions (Harleco product) and Diff-Quik solution (Baxter product) were supplied by the histopathological laboratory of the Michigan State University Clinical Center.

An OLYMPUS AHBS/AHBT research photomicrographic microscope system manufactured by Olympus (Japan) was used for examination of the stained slides. An HFX-DX Labophot-2 three dimension microscope system manufactured by Nikon was used to observe the unstained slides. The photographic film used was Polaroid 52 and 94 supplied by Polaroid Resource Center.

EXPERIMENTAL PROTOCOL

The animals were euthanatized by either cervical dislocation or ethyl ether overdose, and then immobilized on a cork board.

The 22 gauge bevelled aspiration needle was inserted at least 15 mm into the sampling area, and moved forward and back between 35 to 70 times whilst maintaining negative pressure,

i.e. holding the piston at the 8-10 cc mark on the carrier 10 cc syringe. The negative pressure was released slowly, and the aspiration needle removed from the specimen. Ten cc syringes with a syringe-control II, which helps to hold a negative constant pressure, was used for this procedure.

For sample weight determination, the 22 gauge bevelled needle was reweighed after taking the biopsy and compared with the weight before the procedure.

To compare the quantity and quality of the cellular material, a drop of collected material was forced out of the aspiration needle onto a slide, another slide placed on the top of this drop, and the slides pulled apart to make smears. Slides were air dried.

The Rotex needle was used in some cases with a custom instrument holder and at other times without the holder. The needle was rotated to introduce it into the sampling area with the inner screw component completely covered by the cannula. The inner screw part was then rotated out of the cannula into the tissue, rotated clockwise and counterclockwise several times. The cannula was then moved forward over the screw needle completely by a rotating movement, and both components of needle were removed together in one movement. Sample weights were obtained by the same methods as used to with the 22 gauge bevelled needle samples.

To retrieve the Rotex needle sample for evaluation of sample quantity and quality, the inner part of the needle was

pushed out of the cannula immediately, and the tissue material obtained in the grooves of the Rotex needle was rotated clockwise and counterclockwise whilst "smearing" the needle along the glass slide. To remove all of the material from a groove, the edge of a glass slide was placed in the groove, and the screw needle portion rotated counterclockwise onto the glass slide from the one side to the other. The material could be removed completely up to the point of the grooves. The material was then generally air dried. The slides were stained with May-Grunwald-Giemsa stain (Sheehan and Hrapchak, 1980) for cytomorphological evaluation and comparison.

MEASUREMENTS AND OBSERVATIONS

Samples were obtained from 12 different biopsies each of muscle, connective, and adipose tissues from 6 different animals for both FNA and RNB.

The specimen sample weights used with the two biopsy techniques are shown in Table 1-1. The specimens observed from FNA and RNB techniques and tissues for the cytologic evaluations are shown in Table 1-2. As it was difficult to distinguish single myocytes from myofibers, this investigation did not include muscle specimens.

Table 1-1. The specimens used with the two sampling techniques for tissue weight comparison

	FNA	RNB
Connective Tissue	10	10
Adipose Tissue	8	10
Muscle	10	10

Table 1-2. The specimens used with the two sampling techniques in cytologic evaluation

	FNA			RNB		
	T	I	F	T	I	F
Connective Tissue	10	10	10	10	10	10
Adipose Tissue	8	8	8	10	10	10
Muscle	-	-	10	-	-	10

FNA = fine needle aspiration biopsy technique. RNB = Rotex needle biopsy technique. T = total number of the cells present. I = number of cells identifiable as a particular type. F = number of the fragments of tissues or supporting tissue material. - = not counted.

For evaluation of quantity of sample, the cellular material obtained with the FNA and the RNB from connective and adipose tissue were divided into three groups. The "total cells" group was estimated total number of cells present by the number of nucleated cells, excluding leukocytes, regardless of whether or not their type was apparent. The "identifiable cells" group included the number of cells identifiable as a particular cell type. The "tissue fragments" group consisted of the number of conglomerated cells or tissue materials. The specimens were assessed three times for these three groups on each slide to limit counting error.

The cells obtained with FNA and RNB were also observed for their morphological quality, and were classified into five groups: group 0 - nothing found on slides; group 1 - some host material, but with no cells; group 2 - cells present but not diagnosable; group 3 - cells recognizable, but with impaired cellular structures; group 4 - cells present with good sharp images of intact structure. Only cells in groups 3 and 4 usable for diagnosis.

The Student's t test was used to analyze the sample weights obtained from the different tissues with the two types of needle biopsy techniques. In assessing cellular material for quality and quantity, the average and the mean numbers of the total cells present, the identifiable cells, and the tissue fragments were calculated and compared. The Student's

t or Student's t' test was used for assessing significant differences between the numbers of cells or the tissue fragments obtained with FNA or RNB. The numbers of total and identifiable cells were analyzed by Student's t' test. A correlation test was used to analyze the relationship between the number of obtained cells and the number of cellular fragments acquired with FNA and RNB. The Chi-square test was used to analyze the data concerning the quality of cells obtained from the two types of needle biopsy techniques (Steel and Torrie, 1980).

HUMAN MATERIAL

Two hundred nonpalpable breast lesions were examined by mammography, sampled by SFNA and/or SRNB, submitted for pathological diagnosis under standard CAP accredited procedures as part of a regular diagnostic program in the Clinical Center Michigan State University, and were examined retrospectively. The type of needle best providing sufficient cellular material for pathological diagnosis (for both benign and malignant breast lesions) was determined. Sixteen out of the 48 benign lesions, and 6 out of 26 "suspicious for malignancy" lesions did not have clear information about what type of needle biopsy gave sufficient cytopathologic tissues for diagnosis. Therefore, they were not included in this current comparison. All pathological diagnoses were made and reports prepared by board registered pathologists.

Classification of human tissue observations has three groups: "Aspiration only" - the diagnostic material obtained by SFNA; "Rotex only" - the diagnostic material obtained by SRNB; "Both needles" - the diagnostic material obtained by SFNA and SRNB. The Chi-square test was used for analyzing the results obtained from cytopathologic reports under the hypothesis: "Are there significant differences of the sufficient material for diagnosis obtained by SFNA and SRNB" (Steel and Torrie, 1980).

RESULTS

ANIMAL STUDIES

The cytological samples were found to consist mainly of single cells or aggregates of supporting tissues and cells with a varying mixture of blood cells, fibroblasts and tissue fragments. All samples were appropriately stained with May-Grunwald-Giemsa which allowed assessment.

THE WEIGHT OF TISSUES OBTAINED BY FNA AND RNB TECHNIQUES

The weights obtained from muscle, connective tissue and adipose tissue with FNA and RNB are presented in Tables 1-3. FNA samples compared with those of RNB, using the Student's t test, differed significantly for muscle, connective tissue and adipose tissue types ($P < 0.05$). This indicates that the results of tissue weight obtained are significantly different between FNA and RNB for all types of tissues in this study.

Table 1-3. The weights (mg) of samples obtained from animal muscles, connective and adipose tissues

Sample Number	Muscle Tissue		Connective Tissue		Adipose Tissue	
	FNA	RNB	FNA	RNB	FNA	RNB
1	0.4	1.4	1.4	0.8	0.8	1.4
2	0.9	1.5	1.3	1.3	0.4	1.4
3	0.2	1.7	0.9	2.0	0.9	2.2
4	0.3	1.3	0.8	0.6	0.5	1.9
5	0.7	1.2	0.8	1.3	0.8	2.6
6	0.6	1.0	1.1	2.0	1.2	2.6
7	0.6	0.6	1.1	1.8	2.1	2.3
8	0.8	1.0	1.0	1.4	1.8	0.9
9	0.7	0.6	0.5	1.2	1.1	1.8
10	0.7	0.7	1.3	1.0	0.6	1.9
11	0.2	1.8	0.7	2.1	1.1	1.1
12	0.4	1.4	0.4	2.1	0.7	2.0
Means	0.541	1.183	0.942	1.467	1	1.842
SD	0.235	0.409	0.318	0.525	0.510	0.552
SE	0.068	0.118	0.092	0.151	0.147	0.159
p	0.0025		0.0228		0.0056	

SD = Standard deviation. SE = standard error. P = probability value.

The median, maximum and minimum weights obtained from different tissues with FNA and RNB are presented in Figure 1-1. The mean of the weights for all tissues obtained are shown in Figure 1-2.

THE NUMBER OF CELLS AND CELLULAR FRAGMENTS OBTAINED FROM THE DIFFERENT TISSUE TYPES USING FINE NEEDLE ASPIRATION AND ROTEX NEEDLE BIOPSY TECHNIQUES

The averaged total numbers of cells, of the identifiable cells and of tissue fragments obtained in connective and adipose tissue with FNA and RNB present on Table 1-4 and 1-5. Figures 1-3 and 1-4 show graphically the numbers of cells obtained from adipose tissue and connective tissue with FNA and RNB. Figures 1-5 graphically shows the numbers of material fragments from adipose and connective tissues by both needle biopsy techniques.

Results of Student's t test and Student's t' tests analyzing these numbers obtained FNA and RNB from connective tissue and adipose tissue are shown in Table 1-6. All probability values are significant at confidence limits of alpha < 0.05 level.

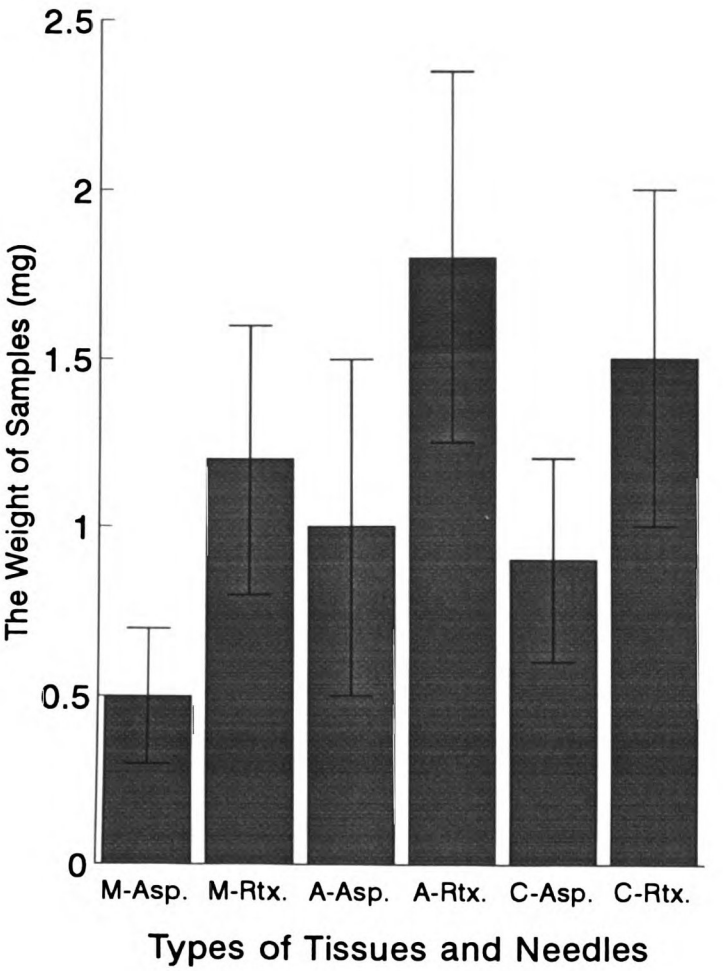

Figure 1-1. The weights (mg) of specimens obtained by the fine needle aspiration biopsy and Rotex needles biopsy technique from different tissue types. (M = muscle, A = adipose, C = connective tissue, FNA = fine needle aspiration biopsy technique, and RNB = Rotex needle biopsy technique). RNB provides higher weight of muscle, connective and adipose tissues ($P < 0.05$ on Students's t tests).

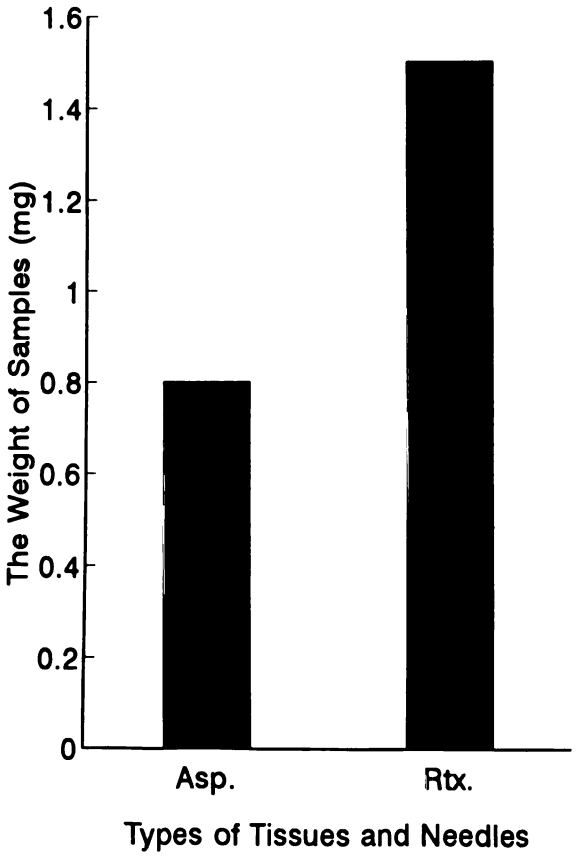

Figure 1-2. The means of the weights (mg) from all types of tissues obtained with the fine needle aspiration and the Rotex needle biopsy techniques. (FAN = fine needle aspiration biopsy, and RNB = Rotex needle biopsy). RNB provides higher mean weights ($P < 0.05$ on Students's t tests).

Table 1-4. The average numbers of cells and cellular fragments obtained from animal connective tissue

No.	FNA Total	FNA Identifiable	FNA Fragments	RNB Total	RNB Identifiable	RNB Fragments
1	5071	2467	5	11683	7949	6
2	32	26	0	8158	6299	15
3	172	58	5	1665	1245	14
4	91	0	2	6371	5887	7
5	763	188	3	3615	2274	2
6	185	49	7	396	315	3
7	541	162	6	4509	3234	4
8	1707	785	20	5199	2545	28
9	811	349	19	5399	3812	33
10	593	344	0	3202	2411	15

Note: FNA = fine needle aspiration biopsy. RNB = Rotex needle biopsy. Total = total number of cells present. Identifiable = numbers of cells identifiable as a particular type. Fragments = the number of tissue fragments obtained.

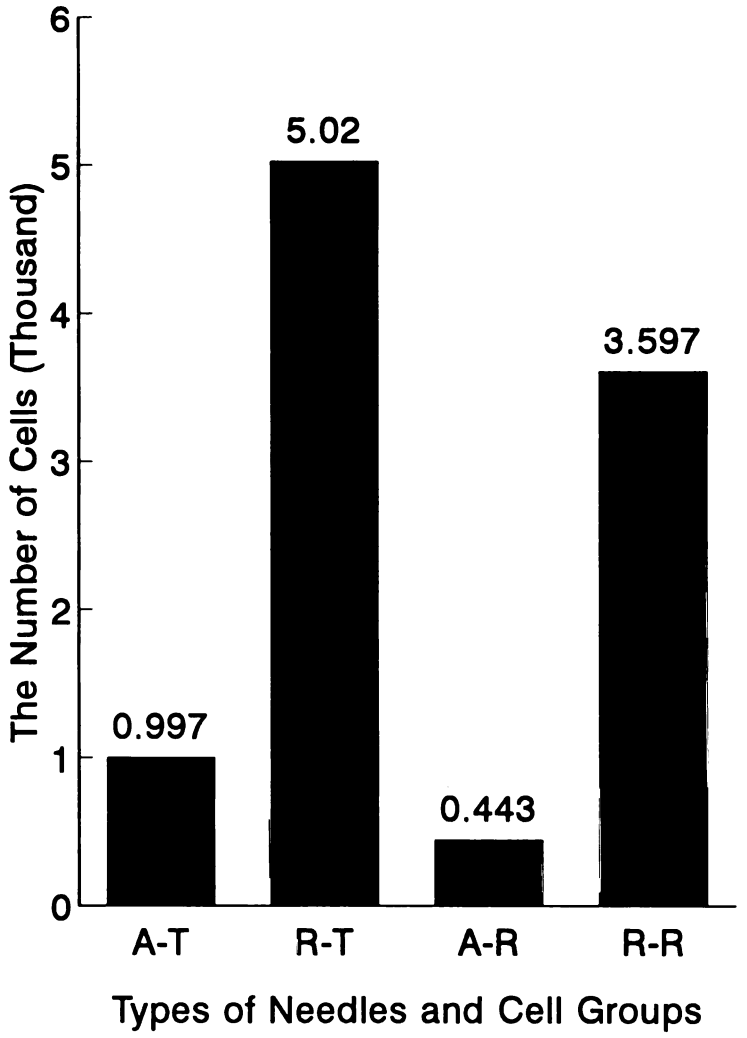

Figure 1-3. The numbers of cells obtained from connective tissue with the fine needle aspiration and the Rotex needle biopsy techniques. (A-T = the total number of cells obtained with the fine aspiration needle biopsy, R-T = the total number of total cells obtained with Rotex needle biopsy, A-I = the number of identifiable cells obtained with fine aspiration needle biopsy, and R-I = the number of identifiable cells obtained with Rotex needle biopsy).

Table 1-5. The average numbers of cells and cellular fragments obtained from animal adipose tissue

Sample No.	FNA Total	FNA Identifiable	FNA Fragments	RNB Total	RNB Identifiable	RNB Fragments
1	0	0	3	130	72	2
2	0	0	1	6644	4335	19
3	1382	161	8	1632	760	6
4	0	0	0	1985	818	21
5	0	0	3	1039	478	18
6	0	0	5	4626	2817	7
7	0	0	9	6713	4060	41
8	0	0	5	195	130	6
9	-	-	-	1551	816	10
10	-	-	-	344	207	4

Note: FNA = fine needle aspiration biopsy. RNB = Rotex needle biopsy. Total = total numbers of cells presented. Identifiable = numbers of cells identifiable as a particular type obtained. Fragments = number of tissue fragments obtained.

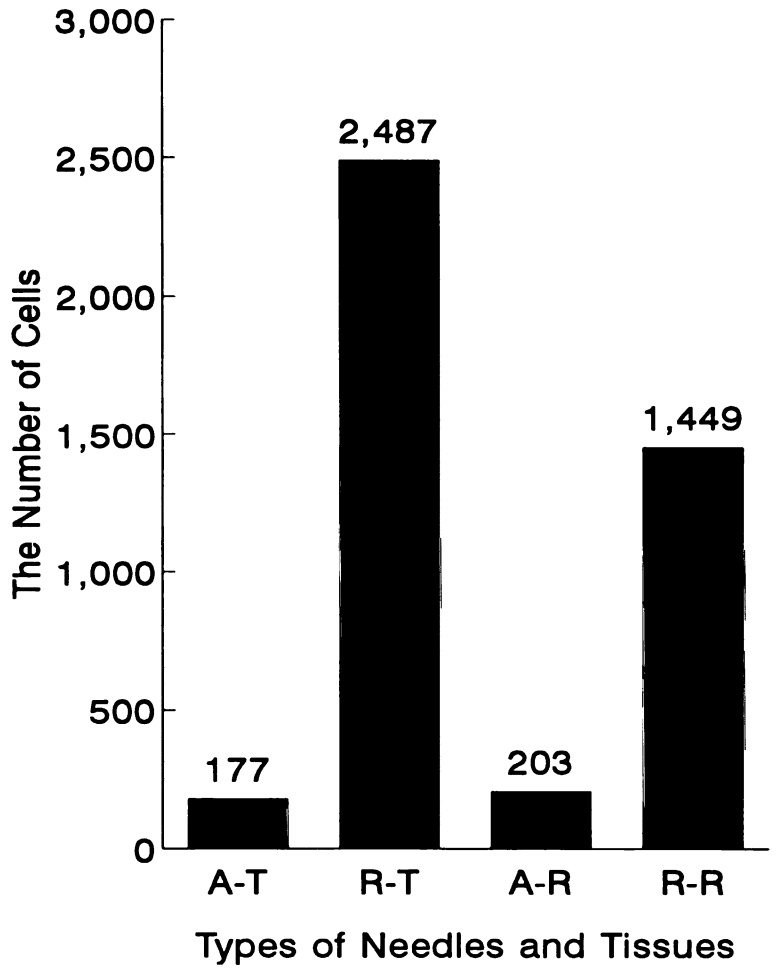

Figure 1-4. The numbers of cells obtained from adipose tissue with the fine aspiration needle and Rotex needle biopsy techniques. (A-T = the total number of cells presented with the fine aspiration needle biopsy, R-T = the total number of cells presented with Rotex needle biopsy, A-I = the number of cells identifiable as a particular type with fine aspiration needle biopsy, and R-I = the number of cells identifiable as a particular type obtained with Rotex needle biopsy).

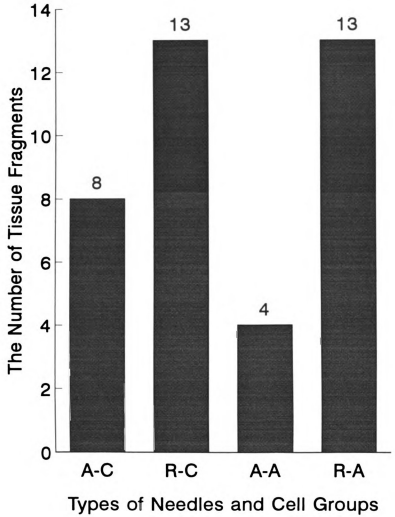

Figure 1-5. The numbers of material fragments obtained from adipose and connective tissues with the fine aspiration needle and the Rotex needle biopsy techniques. (A-C = the fine aspiration needle biopsy on connective tissue, R-C = the Rotex needle biopsy on connective tissue, A-A = fine aspiration needle biopsy on adipose tissue, and R-A = Rotex needle biopsy on adipose tissue).

Table 1-6. Probability of results using Student's t test or Student's t' test on data concerning material obtained with the fine needle aspiration and the Rotex needle biopsy techniques

	Connective Tissue	Adipose Tissue
Total FNA:RNB	0.0006	0.0131
Identifiable FNA:RNB	0.0010	0.0145
Fragment FNA:RNB	0.0286*	0.0319*

*The numbers of material fragments analyzed by Student's t test. The others analyzed by Student's t' test (because of the requirement of the Student's t test assumptions: variances in two group should equal).

Total FNA:RNB = analysis of the total numbers of cells obtained with fine aspiration needle biopsy and Rotex needle biopsy. Identifiable FNA:RNB = analysis of the numbers of identifiable cells obtained with fine aspiration needle biopsy and Rotex needle biopsy techniques. Fragment FNA:RNB = analysis of the numbers of tissue fragments obtained with fine aspiration needle biopsy and Rotex needle biopsy techniques.

CORRELATION BETWEEN THE NUMBER OF CELLS AND FRAGMENTS OBTAINED BY FINE ASPIRATION NEEDLE AND ROTEX NEEDLE BIOPSY TECHNIQUES

The results of the correlation analysis for the results of total, identifiable cells and material fragments obtained are presented in Table 1-7. The correlation between the number of total cells and identifiable cells are significant and linearly related ($P < 0.05$) for all tissue patterns by both needle biopsy techniques. The correlations between the number of total cells, identifiable cells and tissue fragments are significant for adipose tissue by RNB, but not for connective tissue by either FNA or RNB (Steel and Torria, 1980).

THE QUALITY OF CELLULAR STRUCTURE IN SAMPLES FROM FINE NEEDLE ASPIRATION AND ROTEX NEEDLE BIOPSIES

Results of the cell structure differences observed between FNA and RNB are shown in Table 1-8 and Figure 1-6 for different tissues. Chi-square test analysis for diagnosable cell groups (group 3 and 4) show FNA and RNB to be significantly different ($P < 0.05$).

Table 1-7. Probability of correlation test

	Connective Tissue		Adipose Tissue	
	FNA	RNB	FNA	RNB
Total & Fragment	0.649	0.815	-	0.023*
Identifiable & Fragment	0.681	0.959	-	0.037*
Total & Identifiable	0.030*	<.0001*	-	<.0001*

Note: Total & Fragment = analysis on the total number of cells compared with the number of material fragments. Identifiable & Fragment = analysis on the number of identifiable cells compared with the number of material fragments. FNA = fine needle aspiration biopsy. RNB = Rotex needle biopsy. * = significant result with a linearly relationship.

Table 1-8. The results of averaged cell numbers judged for quality with connective and adipose tissues with the fine needle aspiration and Rotex needle biopsy techniques

Type of Tissue Needles	Groups Total #	0	1	2	3	4
FNA-Connective	20	1	2	8	7	2
RNB-Connective	20	0	2	1	3	14
FNA-Adipose	16	4	11	0	1	0
RNB-Adipose	20	4	0	0	4	12

Notes: FNA-Connective = the material obtained with fine needle aspiration biopsy from connective tissue. RNB-Connective = the material obtained with Rotex needle biopsy from connective tissue. FNA-Adipose = the material obtained with the fine needle aspiration biopsy from adipose tissue. RNB-Adipose = the material obtained with Rotex needle biopsy.

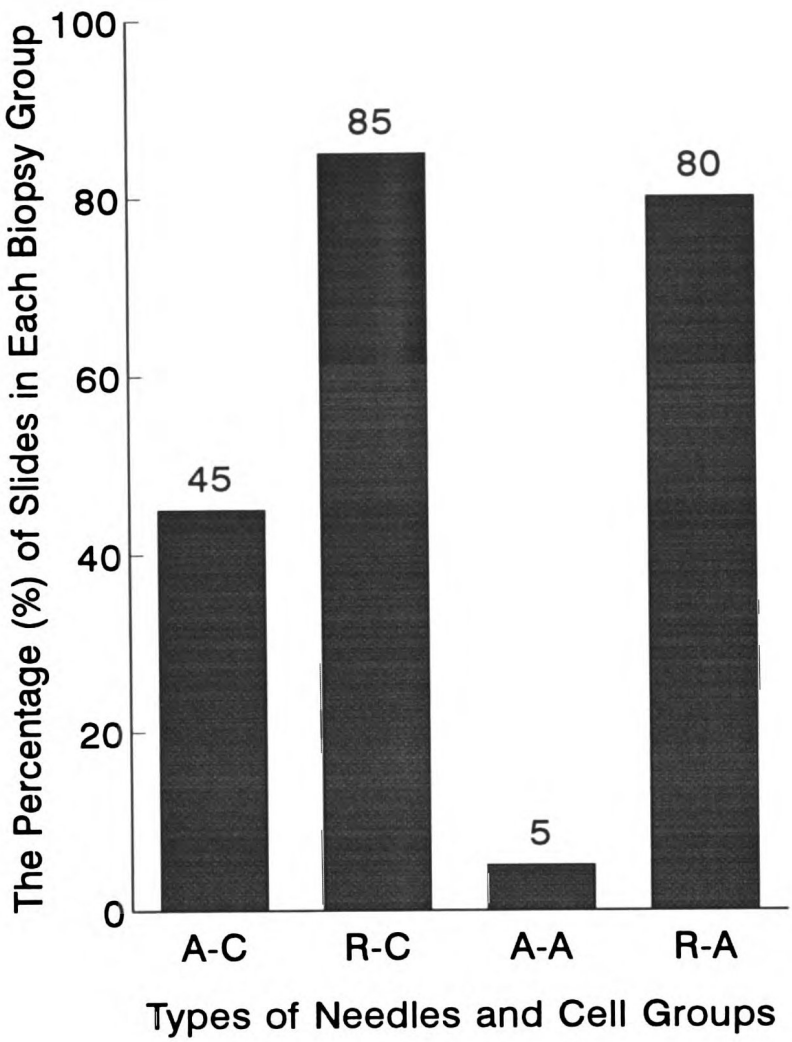

Figure 1-6. The percentages of diagnosable cell groups (group 3 - cells with impaired structure, and 4 - cells with intact structure) obtained with the fine aspiration needle biopsy and the Rotex needle biopsy techniques from connective and adipose tissue. (A-C = the fine needle aspiration biopsy on connective tissue, R-C = the Rotex needle biopsy on connective tissue, A-A = the fine needle aspiration biopsy on adipose tissue, and R-A = the Rotex needle biopsy on adipose tissue). RNB provides better quality of cells than does FNA.

HUMAN PATIENT OBSERVATIONS

Forty-eight benign lesions and 26 suspicious malignant lesions from a base group of 200 non-palpable breast lesions were assessed by SFNA and SRNB. Sixteen of 48 benign lesions and 6 of 26 suspicious malignant lesions were not included in this analysis because of lack of information. Therefore, 32 benign and 20 suspicious lesions were examined. The results of the type of needle producing sufficient information for pathologic diagnosis are presented in Table 1-9 and the percentages are shown in Figure 1-7.

Results of Chi-square analysis show a significant difference ($P < 0.05$) for sufficient cellular material for pathologic diagnosis with both benign and suspicious malignant lesions with either FNA and RNB.

Table 1-9. Number of samples used to examine the cellular material for diagnosis with human tissues

	Benign Lesions	Suspicious Lesions
Total No. Lesions examined	32	20
Aspirate Only	5	2
Rotex Only	14	9
Both Needles	13	9

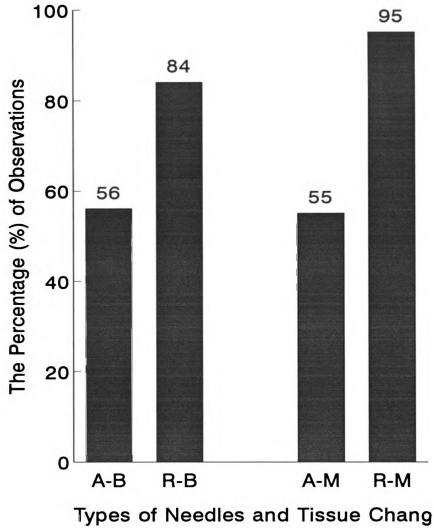

Figure 1-7. The results of biopsy reports indicating "sufficient material" for cytopathologic diagnosis for the fine needle aspiration and the Rotex needle biopsy techniques on human tissues. (A-B = the benign change in the breast was diagnosed with the fine aspiration needle biopsy, R-B = the benign change in the breast was diagnosed with the Rotex needle biopsy, A-M = the suspicious malignant change in the breast was diagnosed with the fine aspiration needle biopsy, R-M = the suspicious malignant change in the breast was diagnosed with the Rotex needle biopsy).

DISCUSSION

This study addresses the questions: Why use the Rotex needle for sampling? Is RNB a better technique than FNA? How much tissue is enough for making a pathologic diagnosis from RNB?

Stereotactic fine aspiration needle biopsy is now a well-established, reliable and safe method for the rapid diagnosis of non-palpable masses, but false negative (11.35 percent) and false positive results (0.17 percent) have been posed as two of the problems with this procedure (Wilkinson, Franzini and Masood, 1991). Results of Nordenström and Azavedo who used fine aspiration and Rotex needles for breast biopsy are very encouraging (Nordenström, 1975, 1977; Azavedo, 1989).

In this study, fine needle aspiration biopsy and Rotex needle biopsy were evaluated based on specimen weights, the number of cells obtained (from animal studies), and sufficiency of material for pathologic diagnosis (from the human tissue observations). Two of three essentials (performing, smearing and interpreting) which affect FNA and RNB results are discussed here.

The weight of material obtained is used to evaluate and compare the capacity of cytological materials with FNA and RNB. The specimen data from muscle, connective and adipose tissues of animals, using the weight of obtained biopsy material, indicate that RNB provides significantly more cellular material than does FNA.

The numbers of cells and tissue fragments obtained by these two types of needle biopsy techniques are significantly different. The RNB provides greater quantity and higher quality biopsy material for diagnosis with connective tissue and adipose tissue of animals and mammary tissue of humans.

Rotex needle biopsy technique not only gives more cells for diagnosis, but preserves the cells' morphology better as well. So this study shows that with the human mammary biopsies, SRNB can provide (44% more with benign lesions and 45% more with suspicious lesions, Figure 1-7 illustrates this showing), more material adequate for diagnosis than does SFNA in this study.

The advantages of RNB over FNA are as follows: 1. Provides a more effective biopsy: RNB with one can obtain a 15 mm long biopsy whereas FNA collects sample only from the tip of needle and its trajectory tract. 2. Rotex needle produces not only single cells, but also some organized structure fragments because of its long cutting edge and the protective cannula cover. In the component of this study addressing adipose tissue biopsy, the FNA does not obtain intact cells as easily as the RNB. 3. A wider range of material can be obtained by RNB, eg. fibrous tissues (elastic fibrous or collagenous fibers); these tissues are hardly obtained by FNA. However, there are some problems inherent to the needle: 1. It cannot be easily used for cysts because it can not hold fluid. 2. Some cellular fragments are too

thick to be evaluated in a smear, reducing the needle's effectiveness.

Interestingly, under specialized microscopy (Nikon HFX-DX or DIC Nomarski Optics) with the unstained fresh biopsy material, many extra findings can be obtained, those shown in, Figures. 1-8, 1-9, 1-10 and 1-11. However, after staining, some of these structures cannot be observed anymore on the slide. These unstained structures have not been used, either for characterization of a diseases or for providing information useful for histopathological diagnosis. Indeed, further studies on three dimensional unstained biopsy materials are necessary.

From our experience, we found some extraneous material (or adherent) fragments that may originate come from the needle and be confused with the host material (see Figure 1-12). To avoid these artifacts, precleaning the biopsy needle, and gently smearing the needle on the slides are recommended.

These are structures that may be useful for making a diagnosis. For example, round structures (macrophage like cells) often occur with cyst lesions (Figure 1-13).

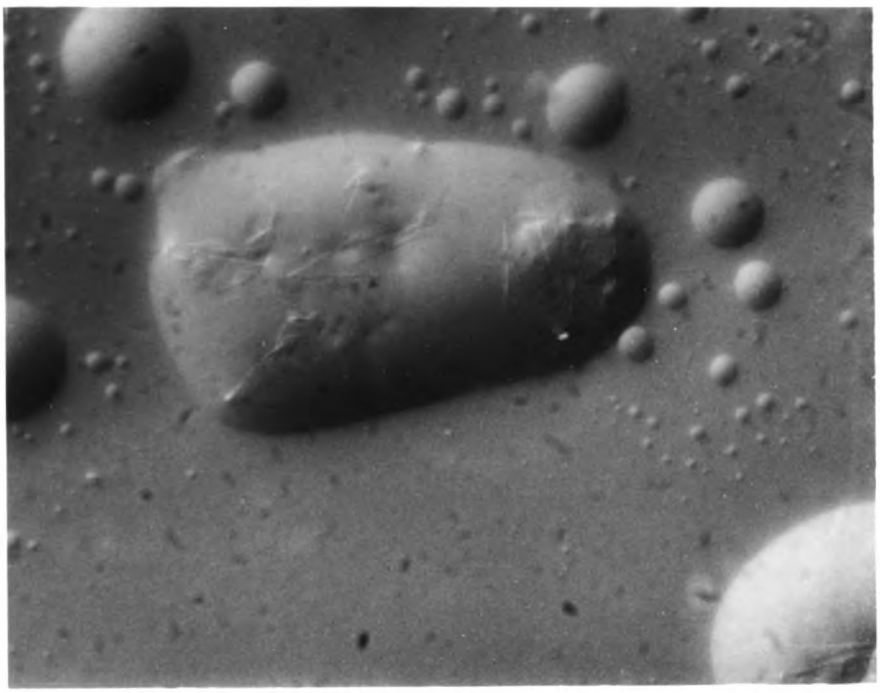

Figure 1-8. An object seen in unstained slide of human breast sample under DIC Nomarski Optics microscopy. After staining the structure can not be observed.

Figure 1-9. An object seen in unstained slide of human breast sample under DIC Nomarski Optics microscopy. After staining the structure can not be observed.

Figure 1-10. An object seen in unstained slide of human breast sample under DIC Nomarski Optics microscopy. After staining the structure can not be observed.

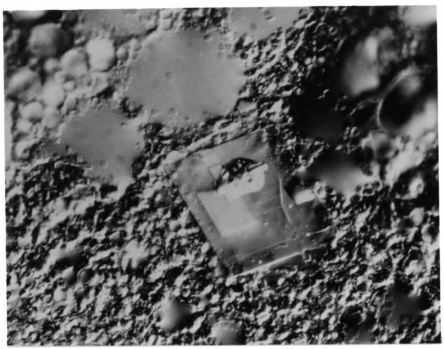

Figure 1-11. An object seen in unstained slide of human breast sample under DIC Nomarski Optics microscopy. After staining the structure can not be observed.

Figure 1-12. The extraneous material fragments originating from needle and contaminating biopsy samples.

Figure 1-13. The appearance of material from a Rotex needle biopsy unstained specimen. It is often found in the fluid from cysts.

How much tissue from a rotex needle biopsy is enough for making a diagnosis? We assessed the correlation between the number of tissue fragments and the number of identifiable cells on a slide, because cellular fragments can be seen at the gross level and may give some indications of the presence of a diagnostic level of cells before the actual microscopic evaluation take place. Results from this analysis shows that the greater the number of total cells obtained, the higher the number of identifiable cells present in connective tissue and adipose tissue samples; this is true for both types of needle biopsy. The numbers of total and identifiable cells obtained are linearly related; however, the correlation between the number of identifiable cells and the number of tissue fragments is variable: with adipose tissue, they are linearly related, but with connective tissue the numbers of cells and tissue fragments obtained are not related to each other. An explanation for this may lie in differences in the character of individual tissue types. The number of fragments that will give an adequate number of cells for diagnosis seems to be 3 - 10 fragments, but the size of fragments, the thickness of specimens and the amount of blood in the background should also be considered (Schnitt and Wang, 1989).

The technique for smearing a Rotex needle specimen was used as Nordenström first introduced it (Nordenström, 1975). This method appears reliable from our experience. However, in this current study, we eventually made smears by when turning

the Rotex needle whilst smearing on the slide. This action involved turning the inner part of the needle by rotating back and forward for the first slides, then turning the needle completely counterclockwise to extrude all the material; this differs from Nordenström (1975) who only turned the needle counterclockwise to make smears. Our approach gave a thinner and more identifiable sample. When using the edge of the glass slide to help to take tissue from the tip of the groove, the lesser the pressure used, the lesser the extraneous contamination from the Rotex needle itself.

There are a number of differing opinions as to how best to make a slide preparation from the needle aspirated material (Schondorf and Schneider, 1978). We chose air-drying with the May-Grunwald-Giemsa stain for animal studies; and air-drying with Diff-Quik staining for human mammary tissue. All slides appeared appropriately stained. These methods are commonly used, simple and reliable.

Our experiences with using the instrument holder with the Rotex needle to perform the biopsy showed that it can hold the Rotex needle in a stable position, allow for easy coverage of the cannula over the inner part of the needle, and allow the whole needle to be easily removed. These advantages shorten sampling time, offer good stability of handling and prevent seeding or implantation of tumor cells into the tissues around the needle track. On the other hand, using the instrument holder requires more working space and limits the angle of

insertion of the needle because of the large size of the instrument. Some experience is also necessary to successfully insert the needle with the instrument holder guiding the Rotex needle.

In conclusion, this study indicated that the Rotex needle biopsy technique is a more suitable, more reliable, and more effective technique for tissue sampling because RNB provides higher quantity and quality of biopsy tissue material than does FNA. It is also diagnostically helpful and confirmatory when used in conjunction with FNA. SRNB can provide 44% on benign lesion and 45% on suspicions lesion more sufficient material for pathologic diagnosis compared with SFNA alone. The analyses in this study predict that the number of fragments could used as a reference for presence of an adequate the number of total and identifiable cells for cellular evaluation on adipose tissue; this may give the SFNA and SRNB user a rough idea of sufficiency of material for pathologic diagnosis. Air-drying and May-Grunwald-Giemsa and Diff-Quik stains are suitable for both needle biopsy techniques. The instrument holder for the Rotex needle could be helpful for the procedure when the user is familiar with its specific characteristics.

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CHAPTER TWO

THE EXPERIMENTAL USE OF DIRECT ELECTRIC CURRENT FOR THE DESTRUCTION OF TISSUE AND A DISCUSSION OF ITS POTENTIAL IN CANCER TREATMENT

BACKGROUND

Surgical, radiological and chemical therapies are the major treatments for breast cancer. Unfortunately, there are many limitation to these therapies. Extensive efforts are underway to develop better methods for cancer treatment. Endocrine, immunological and other approaches have been used experimentally to treat breast malignancy (Fournier and Kubli, 1989; Swain and Lippman, 1991).

Nordenström advanced a new theory which he named the Biological Closed Electric Circuits (BCEC) and developed direct electric current therapy (DC) for treatment of malignant diseases (Nordenström, 1978, 1983 and 1985). The basic principle of the BCEC theory is that the body electric can be compared to a battery, in which the circuit is driven by the separation of oppositely charged ions. In the body, electrical circuits are switched on by an injury, an infection, or a tumor, or even by the normal activity of the

body's organs. Electric currents course through arteries and veins and across capillary walls, drawing white blood cells and metabolic compounds into and out of surrounding tissues and thereby balancing the activity of internal organs and provides the foundation of the healing process. DC treatment can enhance the body's ability to fight tumors. From June 1978, Nordenström began to successfully treat malignancies in patients with DC. This new theory and DC therapy have been followed with interest (Nordenström, 1965, 1977, 1978, and 1983).

The use of DC in medical research has a long history. In 1895 the electrophysiologist Golsinger inserted electrodes with 20 to 40 mA direct current into the brains of dogs and produced focal injuries of tissue (Golsinger, 1908). Horsley and Clarke (1908) reported "for that a unit of time, e.g., 1 minute, there will be about a 1 mm breadth of destruction for each unit of current employed". Ingvar (1920) briefly described reactions of cells to galvanic current using tissue cultures. Louchs (1953) found that cells were more resistant to electrolytic destruction by platinum electrodes than with other metal electrodes. Yasuda used 10 mA to enhance healing of injured bone (Yasuda, 1953). In 1965 the Chinese employed a procedure called "acupuncture narcosis" in a surgical operation which involved the use of a needle carrying a weak DC (Xin, 1990). Malzack described a mechanism for pain control that used an electrode needle carrying DC (Malzack and

Wall, 1965). From 1969 to 1975 O'Conner, Andrews and Backer in different groups studied the effect of DC on bone in vivo (O'Connor et al, 1969; Andrews and Friedenberh, 1970; Backer, 1975). Phillips (1973) made use of alternating current (AC) and DC to produce vascular thrombosis in the control of gastrointestinal hemorrhage. Fukada, Takamatsu and Yasuda reported induction of callus by means of an "electret" (Fukada, Takamatsu and Yasuda, 1975; Yasuda, 1977). By 1979, many experimental and clinical attempts had been made to enhance healing of injured bone by the use of AC and DC (Bassett, Pawlick and Becker, 1964; Brighton, Black and Pollack, 1979; Fukada, Takamatsu and Yasuda, 1975), and a conference on electrically mediated mechanisms of growth in living systems summarized the state of the art in 1974 (Liboff and Rinaldi, 1974).

DC therapy in tumor treatment has been rarely reported. Reis and Henninger reported the effect of DC on Jensen sarcomas in rats and on one patient with vulvar cancer in 1951 (quoted in Nordenström, 1983). In 1967, Gardner, Sterling and Brown studied tumor cells injected into the liver of rats during application of DC and found that the tumor cells were attracted to the anode and repelled by the cathode (Gardner, Sterling and Brown, 1967). Comparable findings of low levels of DC inhibiting tumor growth have been reported by Schanble and colleagues (Schanble, Mutaz and Gallick, 1977). The possibility of using DC in the control of malignant tumors was

raised by Srinivasan in 1977 (Srinivasan, Cahen and Stoner, 1977). In 1979, Bellamy reported that weak current could inhibit the multiplication of cancer cells. Around that time, Nordenström reported his earlier results using DC for tumor therapy and described the first treatment of a human lung tumor with DC (Bellamy et al, 1979; Nordenström, 1983). In 1985, Fu applied a weak current to breast cancers of rats under different conditions and obtained different but effective results (Fu, 1985). Matsushima (1987) combined DC with Bleomycin (an antineoplastic medicine) in vivo in animals, and found that the region of the tumor had higher concentrations of BLM. Because of the difference in electric charges from the drugs, the local concentration of antineoplastic medicines could be different. In 1988 Izuka found that DC therapy performed after radiotherapy produced better results than DC therapy alone or radiotherapy alone (Izuka, 1988). One year later, Quan described DC treatment of 62 patients with malignant tumors, including 9 cases of breast cancers, and reported that effectiveness was 93.5% (Quan, 1989). In 1990, Xin reported the use of DC in treatment of 127 clinical patients with results that demonstrated 77.1% effectiveness. The experimental and clinical studies inducted that DC therapy was safe, effective and simple to perform. It could be a new method for the treatment of malignant tumors (Xin, 1990).

MATERIALS AND METHODS

SPECIMEN

The specimens were fresh bovine muscle obtained from a commercial butcher. The direction of the muscle fibers was vertical to the cut surface of the muscle. The test site chosen was predominantly muscle tissue and was low in adipose and connective tissues.

EQUIPMENT

The TTP No.-1 Baxic Unit (produced by URSUS KONSULT AB, Sweden) was utilized in this study (This machine was provided by Dr. B. Nordenström). This instrument was equipped with two plastic covered platinum electrodes with a 15 mm bare metal terminal at the top of each electrode. The electrodes was 0.2 mm in diameter and 5 mm in length. Continuity of the electrode terminals was confirmed by a "Radioshack" 22-201U Multitester.

The 1-cc syringes were used for fluid collecting. PHydrion paper Dispenser AB 1-11 (supplied by Micro Essential Laboratory Inc.) was used for pH examinations. A general ruler (supplied by C-THRU Ruler Company) was used for local tissue damage measurement. The biopsy specimens were observed under An OLYMPUS AHBS/AHBT research photomicrographic microscope system.

EXPERIMENTAL PROTOCOL

The specimen was flattened on a electric-insulating plate. Electrodes were inserted at a depth of 0.5 cm with distances between positive and negative electrodes of 0.5, 1.5, 3.0 and 8.0 cm. It was not possible to measure the individual affected tissues when the two electrodes were placed at distance of 0.5 cm or closer, so that these are not included.

Different times (5, 10, 20, and 30 min.), and voltages (5, 10, 20, and 24.43 V) were used; 24.43 V was the highest voltage on the machine which we were using), and coulomb levels (5, 10, 20, and 30) were set for each group of experiments.

For the observation of the effects of DC, the sample was incised with a sharp razor blade along the inserted points of the electrodes and along the widest affected area, if it were necessary.

The maximal diameter and depth of each damaged area were measured with a ruler. The fluid in the affected area was collected and measured with the 1 cc syringe. The pH was tested by attaching a pHydrion paper on the treated specimen surface, then comparing with standard markers. The gas production was observed by the formation of bubbles and/or the occurrence of an odor. All measurements during these studies were repeated 3 times and the average numbers were used for evaluation and analysis.

For observing the histologic changes in an affected area, the specimen was fixed immediately in 4% formalin after DC treatment, and the specimen was sectioned and stained with H and E. The tissue processing was performed in the Histochemistry Laboratory of the Michigan State University Clinical Center.

Following DC treatment, the specimens were examined by Magnetic Resonance Imaging (MRI) technique, employing the General Electric 4.7 TOMEGA CSI system. Two sets of images were collected. The first set was obtained with a time of repetition (TR) of 2.0 sec and a time of echo (TE) of 6.0 Oms. For both set of images the field of view (FOV) was 120 mm, slice thickness 2.0 mm, slice separation 3.0 mm and the acquisition matrix was 256 X 128 which was zerofiled to 256 X 256 prior to Fourier transformation.

MEASUREMENTS AND OBSERVATIONS

1. The duration of the gas production.
2. Changes in color and hardness of affected tissue.
3. The maximal diameter of affected tissue around the electrodes either on the surface or in the section sides.
4. The maximal depth of affected tissue (measured by making a vertical sectioning across the affected tissue).
5. The pH of the fluid in the area.
6. The amount of liquid produced during the study, aspirated by 1 cc syringe.

7. The affected tissues were observed by macroscopic examination, and optical microscopy.

8. The characteristics of affected tissues also were observed by MRI.

RESULTS AND DISCUSSION

The fundamental principle of cancer therapy is to change the physiological environment of the tumor to induce cell death. The same basic principle should be addressed with DC treatment. Is the DC treatment safe and dependable? What is the suitable DC voltage for treatment and what occurs at the tissue level during DC treatment? This current study attempted to answer these questions.

The observation of the gas production found that the specimen started to yield gas bubbles and an odor occurred that could be smelled shortly after the two electrodes were inserted into the tissues at the voltage of either 5.00, 10.00, 20.00, or 24.43 V. The generated gas was not measured or examined further. According to Xin (1990) studies: at the positive electrode region, the gas produced is oxygen and chlorine; at the negative electrode region, the gas produced is hydrogen.

The pH on the surface of the electrode in affected areas did not change with the differing voltages, but was different for the positive and negative electrodes. The pH value around the positive electrode was 3 - 4 (strong acidity), and around

the negative electrode was 13 (strong alkalinity). The results showed that the pH at the affected tissues are severely changed during DC treatment. The changes are caused by electrolysis as had been reported by Xin (1990). Hydrochloric acid occurs at the positive electrode and sodium hydroxide occurs at the negative electrode.

The fluid generated during the experiment was collected and measured. Treatment induced considerable amounts of fluid around the positive electrode but comparatively little liquid around the negative electrode. The relationship between the amount of generated fluid and the coulomb level is shown in Figure 2-1. It is a linear relation ($P < 0.05$). The higher the coulomb dose used, the more liquid was produced during the DC treatment. However, voltage increases did not linearly relate in increase fluid with 5 volt producing more fluid than 10 volt level at the same coulomb level (see Figure 2-2). Furthermore, the volume of fluid produced differed with the tissue's natural moisture content.

The relationship between the accumulated coulomb vs. required treating time under varying conditions of different voltages is shown in Figure 2-2. It is clear that by using all the chosen voltages (10.00, 15.00, 20.00, and 23.36 V respectively), the accumulated coulomb is directly proportional to the duration of treatment time. Correlation coefficients and R-square showed there were linear ($P < 0.05$). In other words, increasing the voltage applied between the two

electrodes shortened the treatment time required to reach the desired accumulated coulomb level. These results indicate the optimal time required for DC treatment.

The relationship between the accumulated coulomb and the affected area of tissue is shown in Figure 2-3. Figure 2-3-1 and 2-3-2 show the range of affected tissue around positive and negative electrodes, respectively. In this stage, the affected region was defined as the diameter of the tissues which had changed color due to the treatment. The statistical results showed that coulomb and affected area of tissue was linearly related ($P < 0.05$). Under the experimental conditions of increasing coulomb load, the diameter of affected tissue was continually extended.

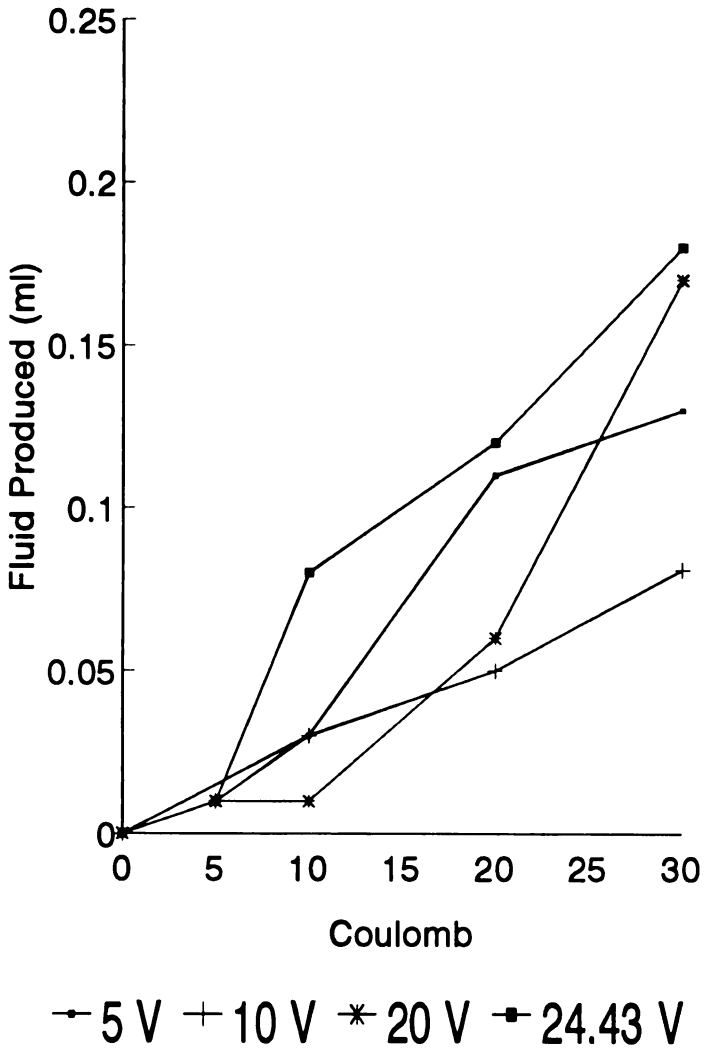

Figure 2-1. The fluid produced at the positive electrode region with different coulomb and volt levels. The higher coulomb used, the more fluid generated.

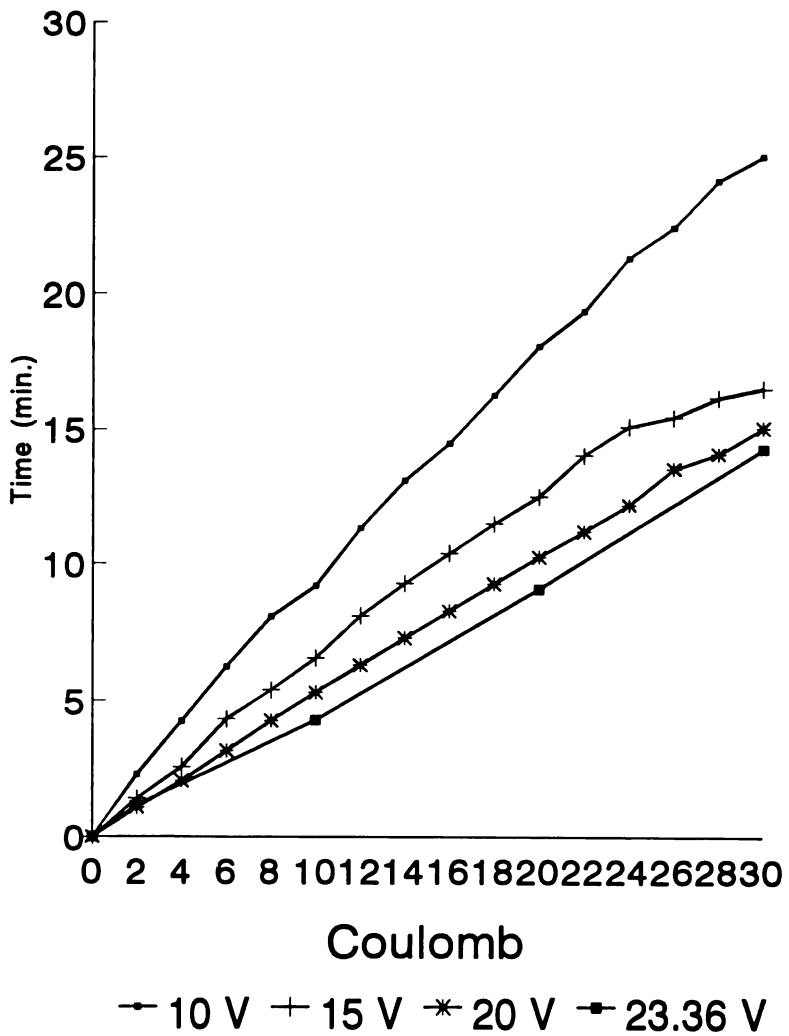

Figure 2-2. The time for development of coulomb with different voltages. (The distance from positive electrode to negative electrode was 1.5 cm). The higher the voltage applied at the two electrodes, the shorter the DC treatment time required to reach a desired accumulated coulomb level.

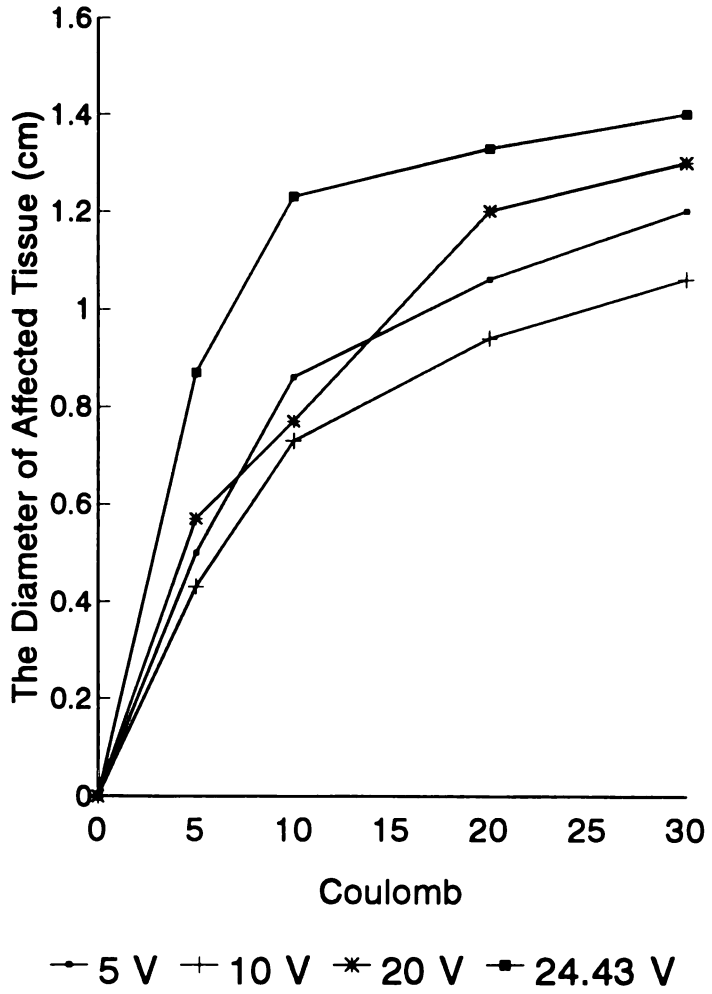

Figure 2-3-1. The correlation between coulomb and the diameter of tissue damage at the positive electrode. (The distance from positive electrode to negative electrode is 3 cm.) The higher coulomb loaded, the greater diameter of tissue affected.

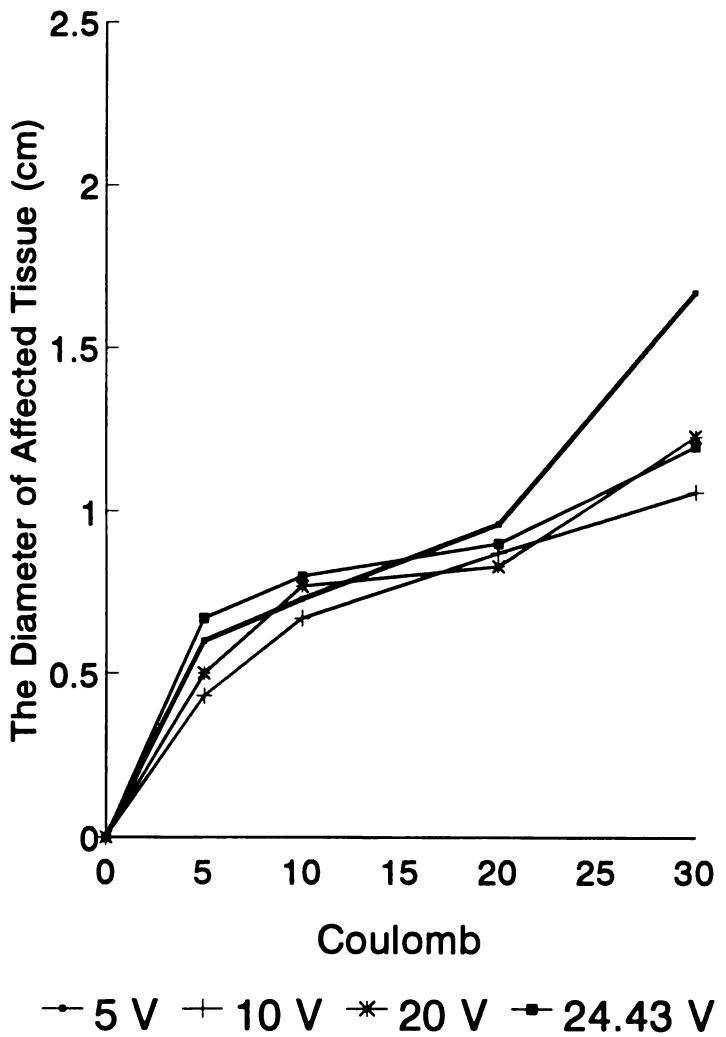

Figure 2-3-2. The correlation between coulomb and the diameter of tissue damage at the negative electrode. (The distance from positive electrode to negative electrode is 3 cm). The higher coulomb loaded, the greater diameter of tissue affected.

The relationship between the voltage and the affected tissue under the condition of 30 coulomb is shown in Figure 2-4. Figure 2-4-1 and 2-4-2 present the diameter of the affected tissues around the positive and the negative electrodes. The data show that the highest voltage (24.43 V) used in this study did not necessarily produce the greatest tissue damage at the negative electrode at the 30 coulomb level. In addition, the lowest voltage (5 V) used did not provide the smallest diameter of tissue damage at both electrode regions. This study suggests that it would be suitable to apply a voltage around 10 to 20 V as standard, because this level of voltage was not only effective but also yielded an appropriate degree of tissue damage.

The depth of tissue damages at both electrodes with different time, voltage and coulomb applications, did not change significantly according to the results of correlation analysis ($P > 0.05$). The probable explanation is that the major element affecting the depth of tissue damage is the depth of the electrode inserted in the specimen.

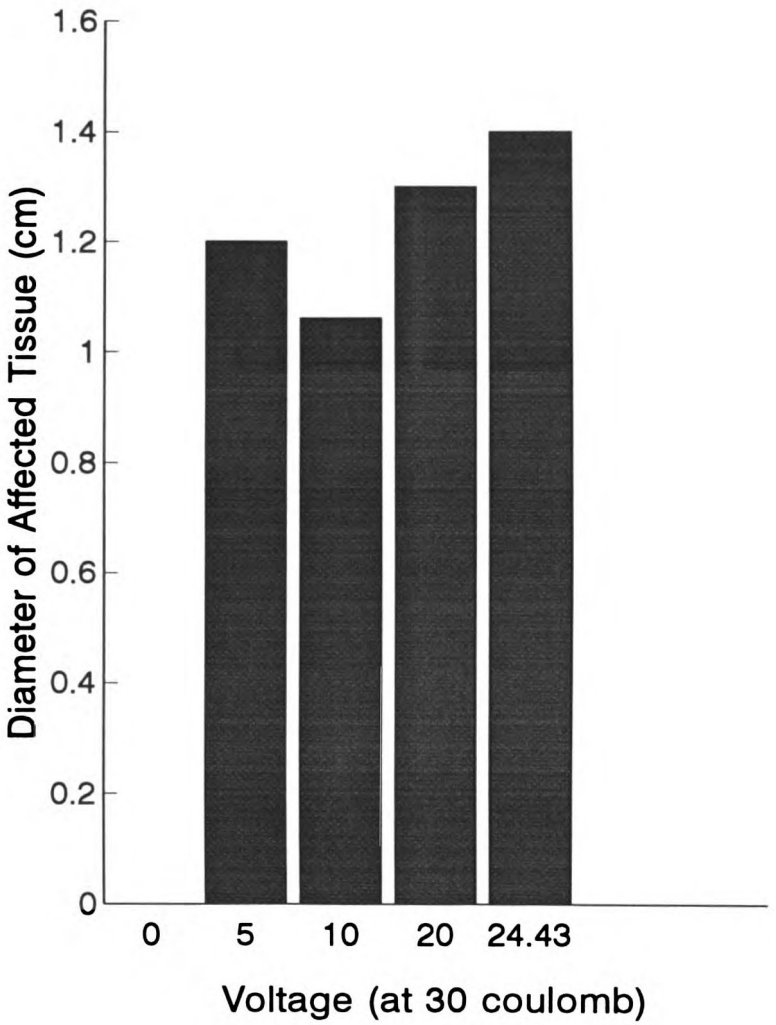

Figure 2-4-1. Relationship between voltage applied and the diameter of the affected tissues at the positive electrode with 30 coulomb level. The lowest voltage (5 V) did not provide the smallest diameter of tissue damage.

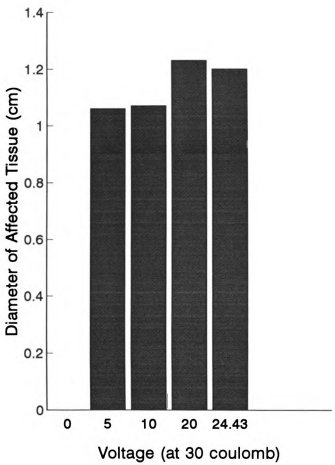

Figure 2-4-2. Relationship between voltage and the diameter of affected tissues at the negative electrode with 30 coulomb level. The highest voltage (24.43 V) did not necessarily produce the greatest tissue damaged at the negative electrode.

The relationship between the distance (1.5, 3.0 and 8.0 cm) separating the two electrodes and the diameter of damaged tissue induced by 10 volt, 20 coulomb at are shown in Figure 2-5. Figure 2-5-1 and 2-5-2 present the diameter and depth of affected tissues respectively. These results imply that under the condition of the same applied voltage, varying the distance between the positive and negative electrodes does not produce significantly different amount of affected tissue area with Student's t test analysis results ($P > 0.05$).

The gross morphological observation indicated that the affected area around the positive electrode turned grey brown with a white center and a dark red jelly-like margin; this affected tissue became firm and had less elastic. The area around the negative electrode turned to a dark red jelly-like substance but remained soft, and no white core was observed.

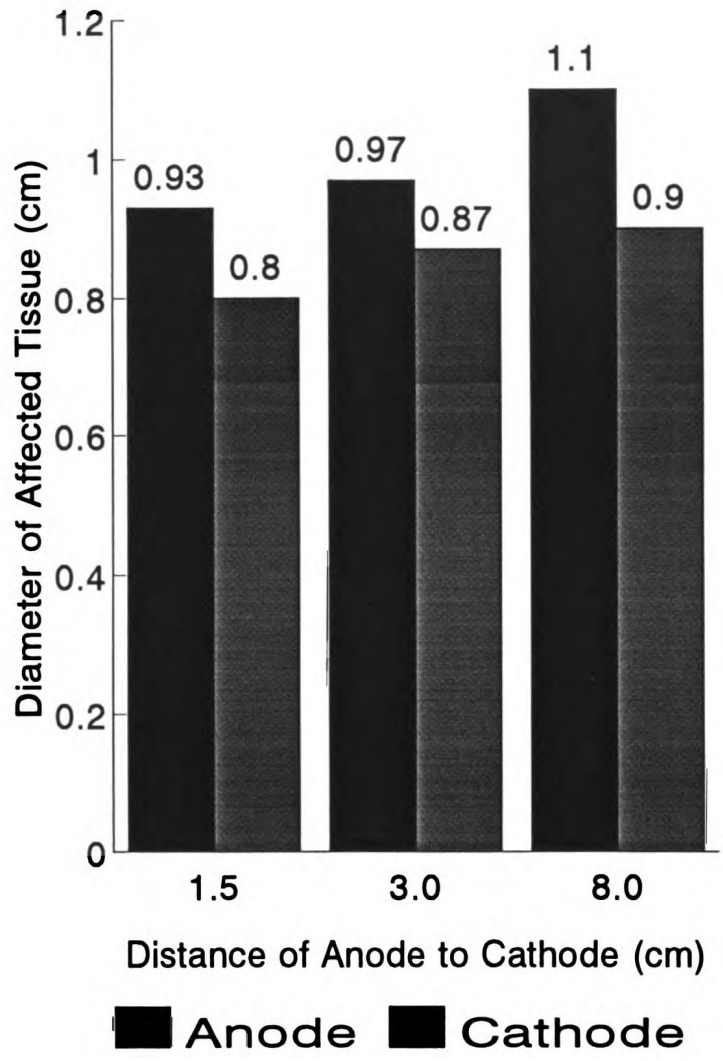

Figure 2-5-1. Relationship between the distance separating the two electrodes and the diameter of the damaged tissue at the positive and the negative electrodes. (All measurements were done at the 10 volt and 20 coulomb level). There are no significant differences in the tissue damage caused by changing the distance of the two electrodes.

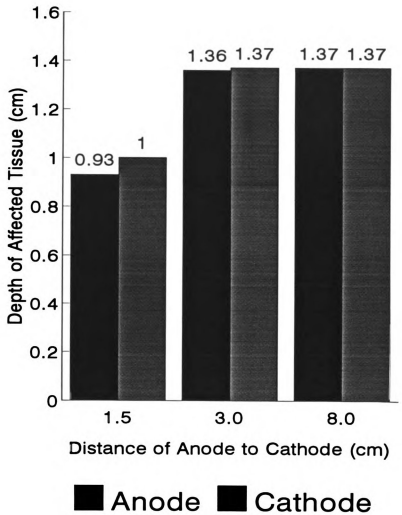

Figure 2-5-2. Relationship between the distance separating the two electrodes and the depth of damaged tissue at the positive and the negative electrodes. (All measurements were done at the 10 volt and 20 coulomb level). There are no significant differences in the tissue damage caused by changing the distance of the two electrodes alone.

The histologic changes in regions at the positive and the negative electrodes were distinctly different. Both affected regions had signs of necrosis. Around the positive electrode, the affected muscle fibers were shrunken and fractured, the cytoplasm lacked homogeneous staining, vacuolations developed, and the striations and the nuclei were absent. The epithelium of blood vessels has degenerated, and the epithelium layer was irregular. Around the negative electrode, the myofibrils were swollen and a homogeneous change appeared in the cytoplasm. The striations and nuclei had disappeared. The mesenchymal space and the blood vessels were narrowed.

The morphological changes suggested that necrosis in tissues occurs at both electrodes. There is no morphological change found between the area of the electrode induced damage. The tissue damage may be caused by local joule heating, severe change in pH, and ions redispersing during the DC treatment. The occurrence of local edema and narrowed blood vessels an changes that could cause the obstruction of blood and oxygen supply to tumor tissue and result in further damage to the local tumor and thereby reduce the risk of metastasis (Xin, 1990; Pasachoff and Kutner 1981).

The MRI image is shown in Figure 2-6. On the anode side, a 1.1 x 0.8 x 0.8 cm, triangle shaped (with the tip downward), increased signal intensity area was noted. The signal intensity was not homogeneous, a vertical low signal line was seen in the middle of the lesion, which probably represented the pathway of the initial insertion of the needle and/or tissue dehydration. The superior part of the increased signal area was protruded out of the surface of the adjacent normal tissue. The changes on MRI represents severe edema with higher protein fluid and/or a higher concentration of hydrogen ion in the tissue of this area.

On the cathode side, a 0.6 x 1.0 x 0.6 cm rectangle shaped high signal intensity area was obtained at the superior aspect of the tissue. The increased signal intensity of this area was less prominent (less brighter) than at the anode. There was a very low signal intensity band which appeared at the center or the lower aspect of the lesion. A low signal linear line extended from the dark band to the surface of the lesion, which is almost as flat as the adjacent normal tissue. These changes on MRI indicate: the high signal intensity region represents an area with more fluid than normal. The low signal intensity region represents an area with severe dehydration. The exact areas of damage caused by DC treatment could be observed.

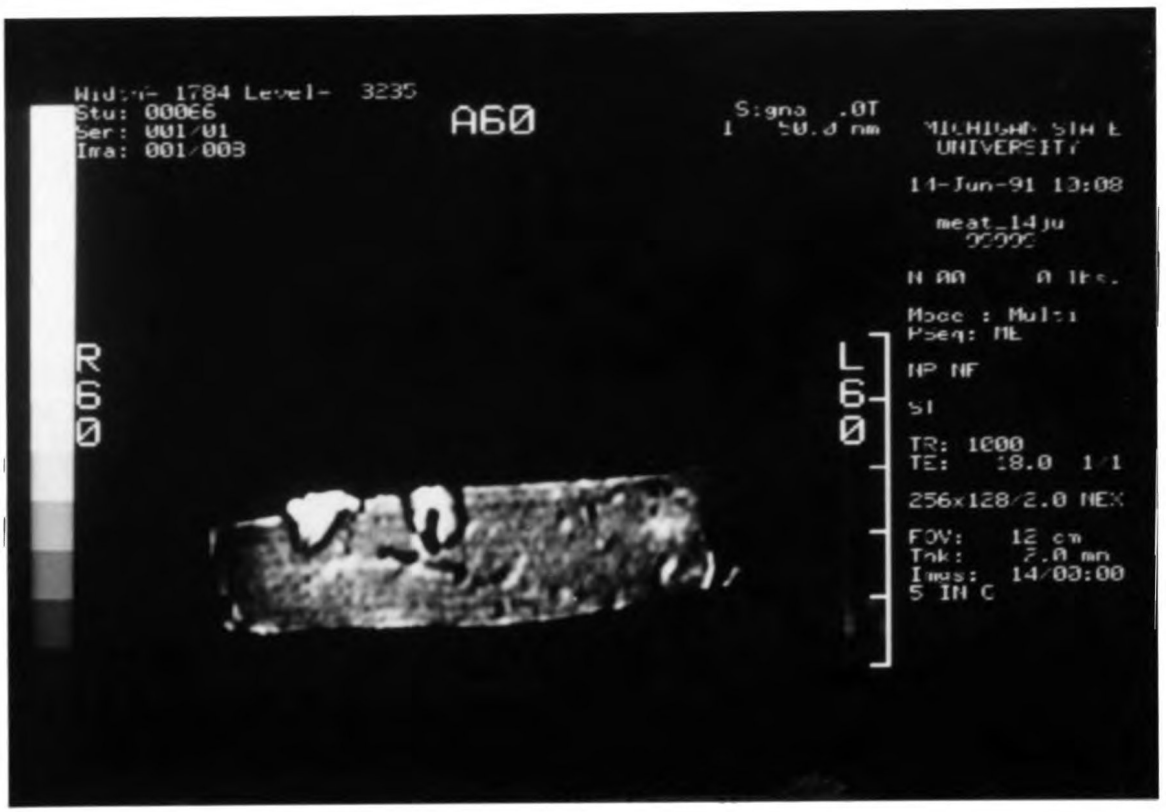

Figure 2-6. The characteristics of Magnetic Resonance Imaging (MRI) in the anode and the cathode regions.

In conclusion, this study indicated that DC is quite likely to be useful as a method for tumor destruction because it can destroy mammalian tissues in a controllable fashion. Considering all the factors (eg. pH changes, fluid production and coulomb/volt loads as well as time) is felt that the 10 to 20 V are optimal to produce the desired degree of tissue damage. By using 10 to 20 V as the suitable voltage value, DC therapy could be a safe, effective and simple procedure. MRI could give a clear indication of the damage area caused by DC treatment. There is a need for further study into the theory of the biological closed electric circuits (BCEC) and its relevance to tumor pathogenesis and treatment.

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