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"One of the most important questions you can ask yourself is, 'am I making the absolute best of this moment?' If you were to make the most of this particular moment, and then do the same in all future moments, life will have a magical way of working itself out."

- Richard Carlson

University of Alberta

Evaluation of autologous platelet gel in breast reduction surgery: A randomized controlled trial

by

Alexander David Anzarut

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of
Master of Science

in

Medical Sciences - Public Health Sciences

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To my wife Jody and son Joshua

Abstract

Introduction: Completely autologous platelet gel (CAPG) is designed to decrease the rate of post-operative seromas and hematomas. We hypothesized the application of CAPG would reduce post-operative drainage and complications, while improving wound healing, compared to standard care in bilateral reduction mammoplasty (BRM).

Methods: We conducted a within-patient, randomized, patient and evaluator-blinded, controlled trial in 111 patients undergoing BRM. CAPG was applied to either the right or left breast. The primary outcome was the difference in wound drainage. Secondary outcomes included pain and wound healing. Assessments of wound healing included size of any open areas, clinical scar assessments, scar pliability, and scar erythema.

Results: No statistically significant differences in the drainage, level of pain, size of open areas, clinical appearance, degree of pliability, or erythema were noted

Conclusions: Our results do not support the use of CAPG in BRM.

Acknowledgements

I would like to thank the University of Alberta Department of Anesthesia, the Caritas Research Fund, EPICORE Centre and Medtronic Inc® for their financial contributions. None of the sponsors had any role in the design, conduct, or interpretation of the study.

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I would like to thank all of the staff at EPICORE Centre, the Misericordia Hospital respiratory therapists, and the University Hospital perfusionists for aiding in the execution of this study. I would like to thank Dr. Tredget for allowing us to use the Mexameter® and Cutometer®. I would like to thank Gloria and the 2D clinic staff for allowing us to use the clinic space. I would like to thank Heather Shankowsky for training and supervising the research assistant. I would like thank all of the surgeons who allowed me to include their patients. This included Drs. Campbell, Edwards, Guiffre, Lobay, Louie, Moysa, Mehling, Morhart, Olson, and Wilkes. I would like to thank Dr. Majumdar for introducing me to the pearls of writing and

clinical research. Finally, I would like to thank Ross Tsuyuki for acting as my primary supervisor.

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List of Abbreviations

Bilateral reduction mammoplasty (BRM)

Autologous platelet gel (APG)

Randomized controlled trials (RCTs)

Platelet derived growth factor (PDGF)

Transforming growth factor beta (TGF-beta)

Vascular endothelial growth factor (VEGF)

Epithelial growth factor (EGF)

Standard deviation (SD)

Milliliter (mL)

Nanogram (ng)

Completely autologous platelet gel (CAPG)

Case report forms (CRF)

Principal investigator (PI)

Epidemiology Coordinating and Research (EPICORE)

Platelet-rich plasma (PRP)

Numerical rating scale (NRS)

Minimal clinically important difference (MCID)

Revised Vancouver Scar Scale (RVSS)

Heather Shankowsky (HS)

Skin elasticity meter (SEM)

Immediate skin distention (Ur)

Final skin distention (Uf)

Erythema index (EI)

Melanin index (MI)

Centers for Disease Control and Prevention (CDC)

Surgical site infections (SSI)

Interquartile ranges (IQRs)

Body mass index (BMI)

1.0 Introduction

1.1 The problem of post-operative fluid collection

One of the most common complications after surgery is the build-up of fluid as either a seroma or hematoma [1-4]. After bilateral reduction mammoplasty (BRM), the incidence of seromas or hematomas is estimated to be between 4-30% and 5-12%, respectively [5]. Fluid build-up can lead to increased pain, infection, poor wound healing, and the need for secondary surgical procedures [6, 7].

Several studies have shown a correlation between post-operative fluid build-up and surgical complications. Hall conducted a prospective observational study to assess characteristics of wound infections after surgery for breast cancer. After following 218 patients, he reported patients with seromas were 6 times more likely to develop wound infections than those who did not develop seromas ($p < 0.0001$)[8]. Varley conducted a prospective trial assessing the use of drains after surgery for femoral fractures[9]. Patients were monitored for the development of post-operative fluid-collection using ultrasound examinations. He demonstrated that patients with increased fluid-build up had significantly poorer wound healing.

1.2 Why suction drains are not the solution

In procedures where post-operative fluid collections are common, suction drains are left in place to allow drainage. However, drains can not resolve all fluid collections[10]. In addition, drains cause patient discomfort, delay discharge from hospital, and provide a route for infection [6, 10-14].

Jani conducted a randomized control trial assessing the effectiveness of drains in preventing seromas among patients having breast cancer surgery[12]. Fifty eight patients were randomized to the drain group and 29 to the control group. The difference in seroma formation rate was not significantly different between the two groups (Drain group: 15/58; Control group: 10/29). Parker conducted a meta-analysis of randomized controlled trials (RCTs) assessing the effectiveness of drains after orthopedic procedures. He was unable to demonstrate a difference in the incidence of hematomas, however, he did demonstrate that patients treated with suction drains were more likely to require blood transfusions[10]. Varley conducted an RCT of drains versus no drains after repair of femoral fractures[7]. He demonstrated a significantly higher rate of wound healing problems in the group treated with drains. Rotstein conducted a prospective study to identify factors associated with wound infections after breast surgery[11]. Four-hundred and eighteen patients were included in the study. It demonstrated a significant association between the presence of closed suction drains and wound infections.

For these reasons, suction drains alone will not solve the problem of post-operative fluid buildup.

1.3 The use of tissue sealants

There is growing interest in the use of tissue sealants to prevent the build-up of post-operative fluid collections. Tissue sealants are classified as either fibrin glues or autologous platelet gels (APG)[15]. The most popular form of commercially available fibrin glue is Tisseel®. Over 1720 surgical centers in Canada and the US are currently using Tisseel®[16]. Although they are commonly used, commercially available fibrin glues are associated with safety concerns. They contain pooled allogenic blood products with the potential for transmission of human immunodeficiency virus, hepatitis B and C, as well as prion disease. Transmission of HIV-1 following the use of a cryoprecipitated fibrin glue has been reported[17, 18].

Autologous platelet gel is an alternate type of tissue sealant. It is made from the patient's own blood and contains no allogenic products. The popularity of APG is reflected by the large number of companies that market equipment for the production of platelet gel. These include Cryoseal FS®, VivoStat System®, SmartPrep System®, Autogel Process Ctomedix®, Haemonetics MCS®, and Magellan Medtronic®. Over 100 surgical centres in the US are using the Magellan Medtronic® system[19].

1. 4 Advantages of autologous platelet gel

The use of APG as a tissue sealant has several advantages. Firstly, it is hypothesized that APGs are hemostatic agents. Autologus platelet gels are said to achieve hemostasis through the formation of a fibrin clot that is initiated by the activation and aggregation of platelets[20, 21]. Secondly, it has been suggested that tissue sealants reduce post-operative pain [22, 23] (see below). Thirdly, APGs contain high levels of growth factors involved in wound healing. These growth factors include transforming growth factor β and platelet derived growth factor [21, 24, 25]. Past research suggests that these growth factors can improve wound healing [26-28] . Fourthly, new technology allows for the consistent production of high quality APG in a variety of settings, including same day surgical facilities. Lastly, when used to cover large surface areas, APGs are more economical. Commercial fibrin glue is sold in aliquots of 1 – 5 mL and when more glue is required the costs increase proportionally. Conversely, the cost of producing APG for a single patient is constant regardless of the volume produced.

1.4.1 Evidence for improved hemostasis with APG

Proponents of platelet gel believe that it is a hemostatic agent. Platelets are known to be a central part the hemostatic process. They release thrombin, thromboxane

A2, and adenosine diphosphate which cause local vasoconstriction and attract additional platelets. In addition, platelets contribute to clot strength.

Laboratory research has shown that platelets are an essential part of hemostatic clot formation. Gottumukala evaluated the independent contribution of platelets and fibrin to clot strength[29]. The amount of force required to break clots produced with and without platelets was compared. He showed that 55% of clot strength was from platelets and 45% was from fibrin.

Human studies assessing the hemostatic potential of APG have been contradictory. Floryan assessed hemoglobin levels at 24 and 48 hours after total knee arthroplasty. He found that patients treated with APG maintained higher hemoglobin levels at both time points than patients in the control group[30]. Man assessed the hemostatic potential of APG in a series of patients undergoing cosmetic surgery[31]. Autologous platelet gel was applied to an area of capillary bleeding and the time to hemostasis was recorded. In all cases (n = 30), hemostasis was achieved 15-45 seconds after the application. Man concluded this was evidence of the hemostatic effectiveness of APG. None of these studies used randomization or blinding of the outcome evaluators.

Other investigators have used post-operative drainage as a surrogate to assess the effectiveness of APG as a hemostatic agent. Castro assessed the effectiveness of

APG in reducing post-operative drainage among patients undergoing transforaminal lumbar interbody fusions of the spine[32]. He did not find a statistically significant difference between the APG treated group and a historical control group (platelet gel: 436mL ± 73; controls: 567mL ± 69, p = 0.29). Wajon assessed the effectiveness of APG and plateletpherisis compared to standard care in reducing chest tube drainage among patients undergoing repeat coronary artery bypass surgery. Outcomes included the cumulative chest tube drainage at 8 hours post-operatively. Eighty-four patients were randomized and no significant differences were found (treatment group at 8 hours: n=40; 407mL ±191; control group at 8 hours: n=44; 411mL ±245; $p > 0.05$)[33]. Neither study reported blinding of the outcome assessors. Although APG is commonly used as a hemostatic agent, there are no evaluator-blinded RCTs to support its use.

1.4.2 Evidence for improved analgesia with APG

It has been suggested that APGs may reduce post-operative pain. Monteleone studied the effectiveness of APG for reducing the pain at split thickness skin graft donor sites[23]. APG treated donor sites had significantly less pain at 7, 14, and 30 days post-operatively. Floryan reported the effectiveness of APG for reducing pain after total knee arthroplasty[30]. APG treated patients (n = 27) had lower mean pain scores than controls (n= 13) (3.6/10 versus 6.3/10; statistical analysis not

provided). In both studies, the authors did not report whether patients were randomized or whether the patients and evaluators were blinded.

1.4.3 Evidence for high growth factor levels in APG

Proponents of APG believe that it improves bony regeneration and soft-tissue healing by delivering supra-physiologic levels of growth factors to the surgical site[21]. The alpha-granules of platelet cells contain numerous growth factors. These include platelet derived growth factor (PDGF), transforming growth factor beta (TGF-beta), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF). Because platelet gel is a platelet concentrate, high growth factor levels are delivered to the surgical site. Using human volunteers, Eppley demonstrated several fold increases in the concentrations of PDGF, TGF-beta, VEGF, and EGF in platelet gel compared to whole blood[21] (Table 1.1). These findings have been independently confirmed[34, 35].

Table 1.1 - Average levels of growth factors in whole blood and platelet gel (adapted from Eppley, B.L., et al. Plastic and Reconstructive Surgery. 114: 1502, 2004).

Growth factor	Concentration in whole blood (ng/mL)		Concentration in platelet gel (ng/mL)		Increase (Mean fold increase)
	Mean	SD	Mean	SD	
PDGF	3.3	0.9	17	8	6.2
TGF-Beta	35	8	120	42	5.1
VEGF	155	110	955	1030	3.9
EGF	129	61	470	317	3.6

N = 10

1.4.4 Evidence for improved bone growth with APG

Several human trials have assessed the effects of APGs on bone growth. Marx published an evaluator blinded randomized control trial to assess the efficacy of platelet gel and cancellous bone graft versus cancellous bone grafts alone to aid in bony growth[36]. This study included 88 patients undergoing reconstruction of mandibular defects. Panoramic radiographs were assessed for graft maturity by blinded observers at 2, 4, and 6 months. At each time point, grafts treated with APG had significantly higher ratings of graft maturity. At 6 months, bone biopsies were taken from the graft site and assessed for levels of bone mineralization using an automated computer imaging system. Grafts treated with APG demonstrated significantly greater bone density. This was the first randomized controlled trial to

demonstrate an increase in bony regeneration with the use of APG. Wiltfang conducted an evaluator-blinded randomized controlled trial comparing the efficacy of tricalcium phosphate with and without APG in patients undergoing sinus lifts[37]. After 6 months, bone biopsies were harvested and histological assessments of bone density were made using computerized image analysis software. The APG group showed a significant increase in bone density compared to the control group.

1.4.5 Evidence for improved soft-tissue healing with APG

The effects of APG on soft tissue healing are less well-defined. Carter used an equine chronic wound model to assess the efficacy of APG to improve wound healing[38]. Wounds were randomized to receive no treatment, saline soaked gauze, or APG soaked gauze. Biopsies of the wounds were taken at 7, 36, and 79 days. Immunohistochemical markers of epithelial differentiation and subjective evaluation of collagen organization in the dermis were used to determine rates of wound healing. The authors reported earlier epithelial differentiation and more organization of the collagen in the APG-treated wounds. No formal statistical analyses were reported.

Zieren used a rat model to compare the effectiveness of polyglycolic acid mesh hernia repair with and without the use of APG[39]. Clinical herniation pressures (the

pressure required to break the wound repair), hydroxyproline concentrations, and the number of fibroblasts and collagen fibers were assessed at 7, 14 and 90 days. There was a statistically significant increase in herniation pressures at 7 and 14 days in the APG treated group. This means the tissues treated with APG were stronger. In addition, the number of fibroblasts and collagen fibers were statistically higher in the APG treated animals. The authors did not report whether the animals were randomized or whether the evaluators were blinded.

Monteleone studied the efficacy of APG for accelerating the rate of re-epithelialization of split thickness skin graft donor sites[23]. Each of the 20 study patients had two donor sites. One site was treated with bovine thrombin and the other with platelet gel. Re-epithelialization was assessed using photographic analysis. Outcomes were assessed at 7, 14, 20, and 30 days post-operatively. APG treated donor sites had significantly more rapid re-epithelialization at each time point. The authors did not report whether patients were randomized or whether the evaluators were blinded. These findings were presented as an abstract in 2000 and have not been published as a full article.

Senet published a double-blind, placebo controlled, randomized trial assessing the efficacy of a APG for the treatment of chronic venous ulcers[40]. Patients were evaluated every 4 weeks with standardized digital photography. The mean rate of wound healing in the treatment group ($n = 7$; 0.0033 ± 0.0061 cm/day) was not

statistically different from that of the control group (n = 8; 0.0021 $\pm$ 0.0058). This study was not adequately powered and would have required several hundred patients to avoid making a type II error.

Mazzucco reported the results of a pilot study designed to assess the effectiveness of APG in the treatment of dehiscent sternal wounds and necrotic skin ulcers[41]. Endpoints for the dehiscent sternal wound patients included time to complete wound healing and total length of hospital stay. Endpoints for the necrotic skin ulcer patients included time necessary to achieve a healthy wound base suitable for surgery. Compared to the control group, the platelet gel treated dehiscent sternal wounds healed more quickly (3.5 versus 6.0 weeks; $p = 0.0002$) and were discharged home earlier (3.5 versus 52.5 days; $p < 0.0001$). APG treated necrotic ulcers developed a healthy wound base earlier than the control group (15 versus 35.5 weeks, $p < 0.0001$). Blinding and randomization were not performed in this study.

Adequately powered and well designed randomized controlled trials are needed to address whether APG can improve soft-tissue healing. Although numerous studies suggest that APGs improve soft-tissue healing, there are no peer-reviewed human studies to support this[31, 42-47].

1.5 The safety of currently available autologous platelet gels

Although autologous platelet gels may have potential benefits, their use is associated with safety concerns. Autologous platelet gels are made with bovine thrombin. There are several reports of anaphylaxis after exposure to bovine products[48-53]. In addition, exposure to bovine products can induce formation of auto-antibodies against fibrinogen, factor V, and thrombin. Such reactions have lead to life threatening coagulopathies[54-56]. Finally, there is a theoretical risk of transmitting of prion disease. Despite these concerns, the use of platelet gel in surgery is increasing.

1.6 Completely autologous platelet gel

In response to the concerns surrounding bovine products, technology was developed to allow for the production of completely autologous platelet gel (CAPG). This became available in January of 2004. Completely autologous platelet gel is made with only the patient's own blood and the addition of citrate. Because there are no allogenic human or animal components, this is much safer than commercially available fibrin glues and traditional platelet gels.

CAPG is a platelet-based wound sealant that is produced from centrifugal separation of whole blood. This process creates a platelet concentrate. A small

portion of the platelet concentrate is exposed to sterile glass, resulting in activation of the clotting cascade and the production of a fibrin platelet mixture. The actual platelet gel is produced at the time of application when the platelet concentrate is mixed with the fibrin platelet mixture.

Currently there is very little literature available assessing CAPG. A literature search was conducted of the following computerized bibliographic databases: MEDLINE (1966-present), EMBASE (1988-present), CINAHL (1982-present), PEDro (Physiotherapy Evidence Database), Cochrane CENTRAL Register of Controlled Trials, Web of Science and Dissertation Abstracts. The search terms included: "PRP," "platelet gel," "platelet rich-plasma," and "tissue sealants." In addition, the reference lists of all potentially relevant articles were reviewed. Finally, Medtronic Inc. was contacted to identify the existence of any human trial assessing CAPG. Although CAPG is currently available for use in Canada, we were unable to identify any trials assessing its efficacy.

1.7 Bilateral reduction mammoplasty (BRM) to test the efficacy of CAPG

Bilateral reduction mammoplasty (BRM) patients provide the ideal setting to test the efficacy of CAPG. BRM is the most common operation performed by plastic surgeons. In 2003, plastic surgeons in Edmonton performed 798 BRMs[57]. The problem of post-operative fluid collections after BRMs is clinically significant and

common. As previously stated, hematomas and seromas are the most common complications after BRM and are estimated to be between 4-30% and 5-12%, respectively[5]. Their occurrence can contribute to wound infections, poor wound healing, the need for secondary operative procedures, and tissue necrosis.

Studying BRM patients allows for a paired research design[58]. In each patient, one breast can serve as the intervention side and the other as the control. The use of a paired design reduces between subject variability and allows for a smaller sample size.

1.8 Hypothesis

The subcutaneous application of completely autologous platelet gel during BRM surgery will reduce the amount of post-operative wound drainage and post-operative pain; improve scar quality; and decrease the frequency of hematomas, seromas, infections, and wound healing complications, compared to no treatment.

1.8 Specific objectives

The primary objective was to assess the efficacy of CAPG in reducing post-operative wound drainage after BRM.

The secondary objectives were:

1. To assess the efficacy of CAPG in reducing post-operative pain.
2. To assess the efficacy of CAPG in decreasing the size of open wounds.
3. To assess the efficacy of CAPG for in improving of the clinical appearance of the scar, as measured by the Revised Vancouver Scar Scale and the Beausang Scar Scale.
4. To assess the efficacy of CAPG in improving scar colour, as measured by the Mexameter®.
5. To assess the efficacy of CAPG in improving scar pliability, as measured by the Cutometer®.
6. To assess the efficacy of CAPG in reducing post-operative complications.

2.0 Methods

2.1 Overview of the study design

The evaluation of completely autologous platelet gel in breast reduction surgery: A randomized controlled trial was a prospective, randomized, within patient, controlled trial. Bilateral reduction mammoplasty (BRM) patients were randomized to receive platelet gel to either the right or left breast. The contralateral breast received no treatment. Patients were followed for six weeks to assess the effects of platelet gel on wound drainage, pain, wound healing, and post-operative complication rates.

2.2 Patient population

2.2.1 Inclusion criteria

All subjects were females at least 18 years of age, scheduled for BRM, and staying within a one hour drive of the hospital during the first 24 hours post-operatively.

2.2.2 Exclusion criteria

Patients were excluded from the study if any of the following were present: (1) prior history of breast surgery (excluding BRM) (2) history of coagulopathy (3) antiplatelet agent use within 10 days of surgery, (4) language barrier, (5) no access to a telephone, (6) previous enrollment in the study, (7) or an unwillingness to return for follow-up. Exclusion criteria for all patients were recorded on the case report forms (CRFs) (Appendix 1).

2.3 Setting

Volunteers were recruited from the practices of 11 plastic surgeons. This included all plastic surgeons performing BRMs in Edmonton, Alberta, Canada. Surgeries took place at the University of Alberta, the Royal Alexandra, and Misericordia Hospitals. This represents all public surgical facilities performing BRM surgery in Edmonton. Eight of the surgeons had a minimum of 10 years experience and had each performed over 100 BRM prior to their involvement with the study. The remaining three surgeons each had greater than 2 years experience. The plastic surgeons in Edmonton provide services to patients throughout Northern Alberta, Northern British Columbia, Nunavut, the Yukon, and the Northwest Territories.

2.4 Ethics

The study protocol, consent forms, and patient information sheets were approved by the University of Alberta Health Research Ethics Board and the Community Research Ethics Board of Alberta (Appendix 2 - Ethics). All data was sent to the research coordinating office with study numbers and patient initials only. Any information that included patient identifiers was kept by the principal investigator only.

2.5 Baseline data collection

2.5.1 Pre-operative demographics

Information on date of birth, weight, height, chest circumference, cup size, smoking history, steroid use, and diabetes was collected (Appendix 1 – CRFs). This data was collected by the principal investigator (PI) pre-operatively during the screening telephone call and was confirmed on the morning of the surgery.

2.5.2 Intra-operative data

Immediately after the surgery, the PI used the operating room data sheet from the patient chart to collect the intra-operative data (Appendix 1 – the operative room

data CRF). This data included the anesthesia start time, the time the patient left the OR, the amount of breast tissue removed from each side, the type of breast reduction, the surgeon's use of pre-operative infiltration, the amount of pre-operative infiltration, liposuction, the amount of liposuction on each side, the involvement of a resident in the resection and the breast side that the resident operated on.

2.6 Randomization and treatment allocation

Randomization and treatment allocation was done through an independent data management centre (the Epidemiology Coordinating and Research (EPICORE) Centre, University of Alberta). A computer-generated random numbers program was used to produce the randomization sequence. Randomization was done in blocks of 4 to ensure that a similar number of right and left breasts received treatment. Investigators were not made aware of the block size. Randomization was stratified by surgeon and by centre. This allowed us to control for surgeon and site as potential confounders. Treatment allocation was through the use of sequentially numbered opaque sealed envelopes. Randomization envelopes were opened after resection and hemostasis was complete for both breasts. This ensured that surgical hemostasis would be equal on both the treatment and control sides.

2.7 Intervention: Production and application of the platelet gel

2.7.1 Blood collection

The anesthetist collected 52mL of the patient's blood into a 60mL syringe. This was done prior to making any surgical incision to avoid platelet activation. A 16 gauge needle was used to avoid platelet lysis. The syringe contained 8mL of citrate- dextrose anticoagulant (Cytosol Laboratories, Inc. Braintree, MA, USA) to prevent the blood from clotting. Samples were gently rotated through 360 degrees to mix the anticoagulant.

2.7.2 Generation and application of the CAPG

The sample was then placed into a dual speed centrifuge (Magellan®, Medtronic, Inc., Minneapolis, MN, USA) for 17 minutes (Figure 2.1 – photo of Magellan unit). This separated the platelet-rich plasma (platelets and white blood cells suspended in plasma) from the red blood cells and the platelet-poor-plasma. This resulted in 8mLs of platelet-rich plasma (PRP). Of this, 5mLs was drawn up into a syringe and the syringe was connected to a plastic dispensing device. The remaining 3mLs of PRP were transferred to a syringe containing sterile woven glass (Figure 2.2 – syringe with woven glass). Exposure of the PRP to the glass for

10 minutes resulted in activation of the platelets and release of thrombin and calcium into solution. The activated PRP was subsequently transferred to a second syringe and this syringe was also connected to the plastic dispensing device. As the syringes were depressed, the Y- tip at the end of the dispenser resulted in mixing of the PRP and activated PRP (Figure 2.3 – dispensing device with the Y-tip end). This final step led to the creation of the platelet gel. After randomization and prior to surgical closure, the gel was applied topically to the subcutaneous tissues of either the right or left breast by the attending surgeon under the supervision of the PI. No placebo substance was applied to the control breast.

Figure 2.1 – The Magellan® centrifugation system (Magellan, Medtronic, Inc., Minneapolis, MN, USA)

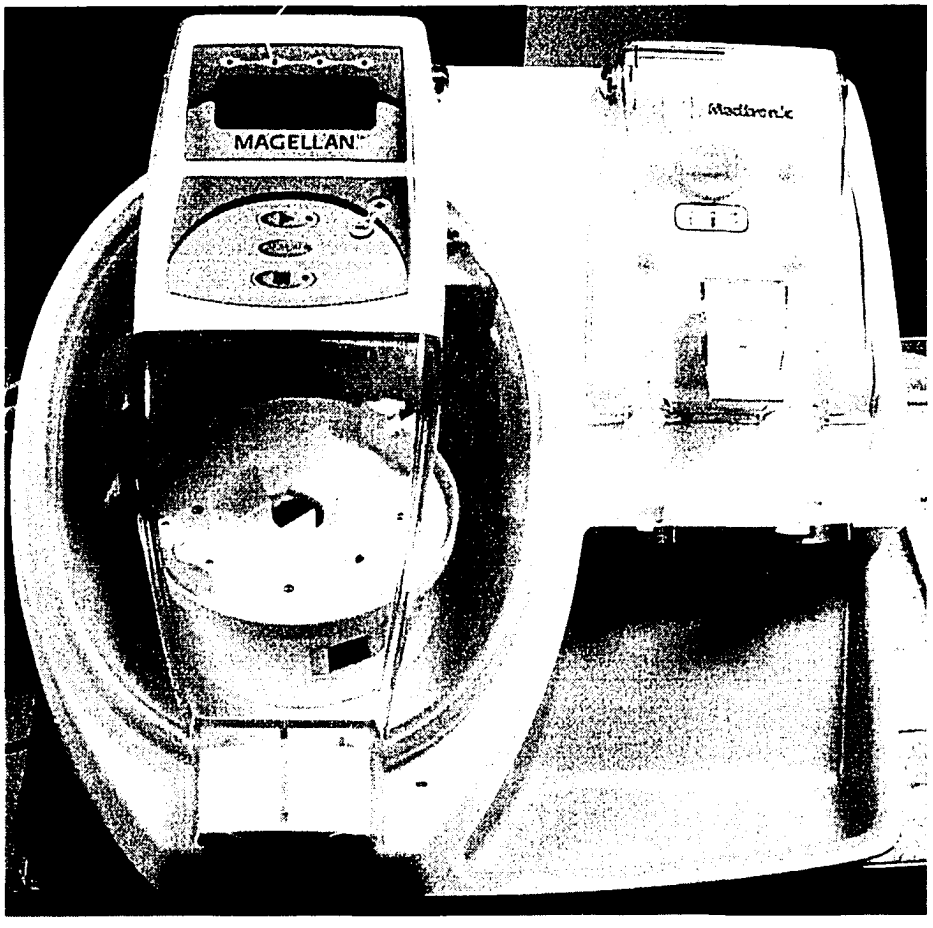


Figure 2.2 – Syringe with woven glass

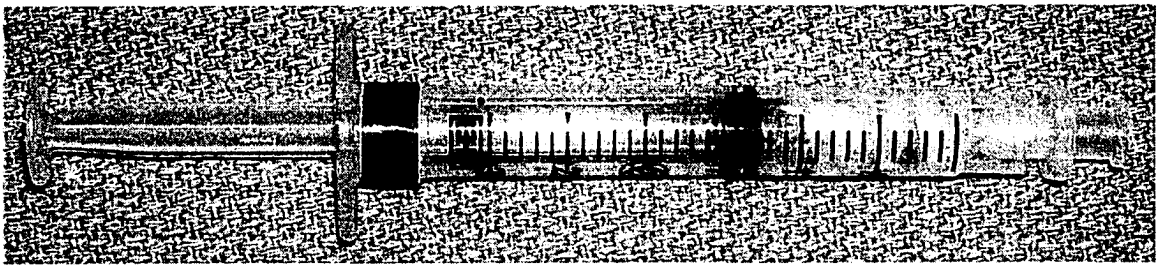
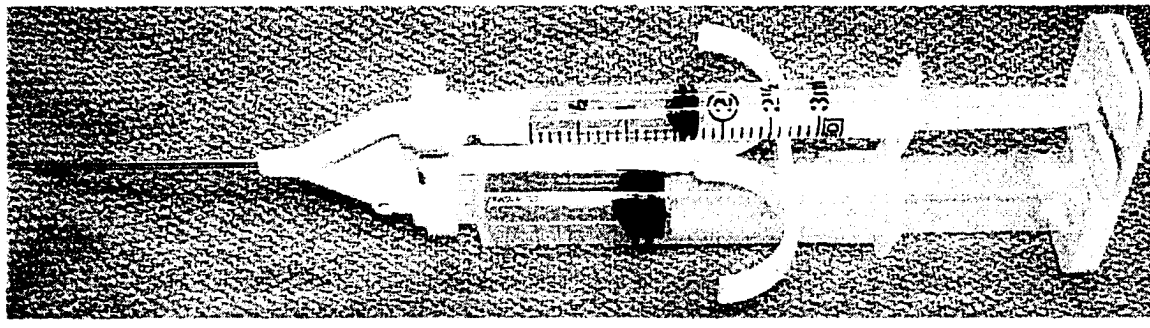


Figure 2.3 – Dispensing device with the Y-tip end



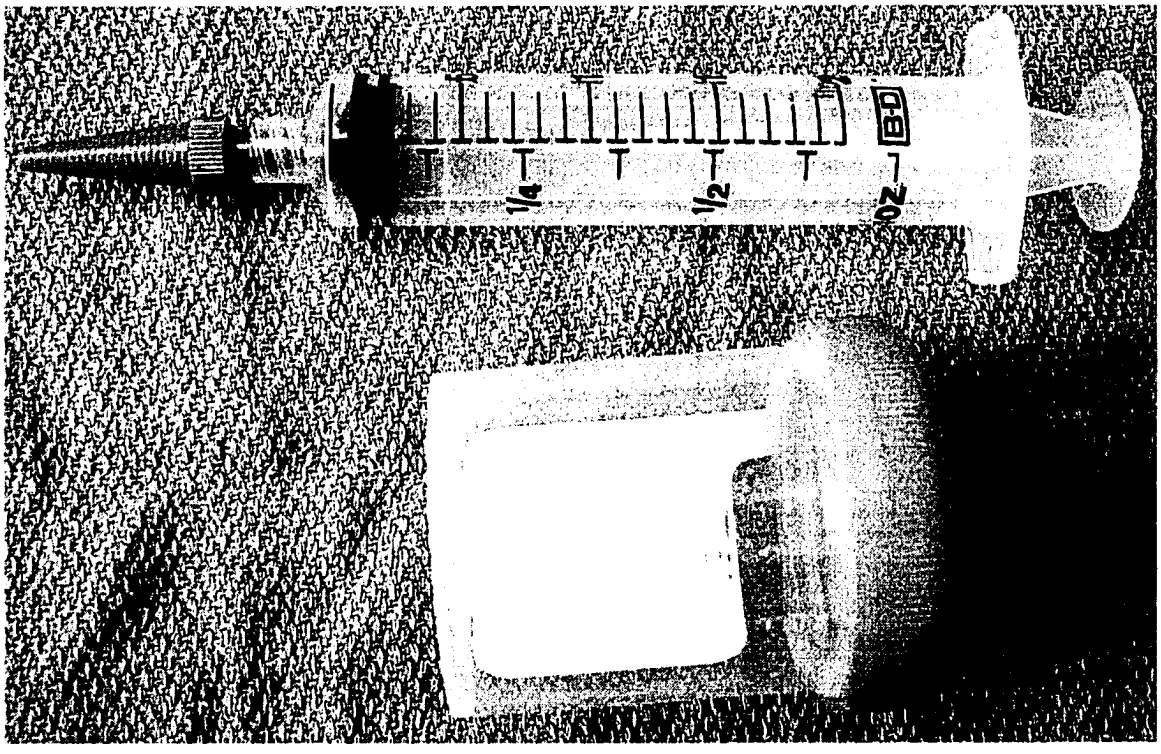
2.8 Outcome measures

2.8.1 Primary outcome: Post-operative drainage

The primary outcome measure was the difference in post-operative drainage between the treated and control breasts over the first 24 hours. Increased wound drainage has been correlated to increased rates of post-operative seromas, hematomas, infections, and poor wound healing[12, 59-61]. Jackson-Pratt 7 mm fully perforated ¾ inch drains were placed in each breast at the end of surgery. A research nurse, blinded to patient allocation, emptied each drain a minimum of every eight hours for the first 24 hours post-operatively. For patients admitted to hospital post-operatively, this was done on the ward. For patients having same day surgery, research nurses drove to patients' homes to make the assessments.

Contents of the drain were emptied first into a urine specimen container and then aspirated into a 20 mL syringe with a catheter tip adapter (Figure 2.4 – equipment used for drain measurement). The use of a graduated syringe to measure drain outputs allowed for more precise measurements.

Figure 2.4 – Equipment for drain measurement: Syringe (20 mL) with catheter tip adapter



2.8.2 Secondary outcome measures

The secondary outcome measures included pain, assessment of the size of any open areas, clinical assessments using comprehensive scar scales and automated measures of scar colour and pliability.

2.8.2.1 Numerical Rating Scale (NRS) for pain

A numerical rating scale (NRS) was used to assess the intensity of post-operative pain. Pain intensity is the most clinically relevant dimension of pain[62]. The NRS is easily understood by patients and is associated with high levels of compliance[63-65]. It is reliable[66-68] sensitive to treatment effects[69] and is a valid method for the assessment of post-operative pain[70]. The 0 – 10 NRS is has been recommended for use in pain related outcomes research by both the Emergency Medical Services Outcomes Project and the European Association for Palliative Care[70, 71].

A score of 4-6/10 corresponds to moderate pain intensity, while scores of ≥8/10 correspond to severe pain intensity[72-75]. In patients with moderate post-operative pain, the minimal clinically important difference (MCID) is 1.3 (95% CI 1.2-1.4), or a change of 20.1% (95% CI 18.1-22.2) from baseline[75].

Pain was assessed for each breast using a 0-10 NRS, with 0 representing no pain and 10 representing the worst pain (Appendix 1 – the NRS). The scale was administered by a research nurse every 8 hours for the first 24 hours post-operatively. The average pain score over the first 24 hours post-operatively was calculated for each breast. In addition, a research assistant assessed each breast at the 1, 3, and 6 week follow-up visits.

2.8.2.2 Open areas

Areas that had not re-epithelialized at 1, 3, and 6 weeks were considered open. The average length and width of each open area was used to calculate the area of skin remaining open. At the 1, 3 and 6 week follow-up visits, the research assistant measured the size of open areas using a ruler with one millimeter graduations.

2.8.2.3 The Revised Vancouver Scar Scale

The Revised Vancouver Scar Scale (RVSS) is a comprehensive clinical rating scale for the assessment of scars (Appendix 1 – the RVSS). The scale includes pliability, pigmentation, height, and vascularity[76]. Each of these values is given a score of between 0 and 3 or 4; increasing values indicate more severe scarring. The individual parameter scores are added to give an overall score for each scar. Scores range from 0 to 14, with low scores representing clinically well-healed

scars. The RVSS is based on the Vancouver Scar Scale which has demonstrated adequate reliability and construct validity[76-78].

The research assistant responsible for the RVSS assessments was trained and supervised by a nurse (Heather Shankowsky)[76, 79] with expertise in wound healing. Assessments were performed on both breasts at the 3 and 6 week follow up appointments.

2.8.2.4 The Beausang Clinical Scale

The Beausang Scale is a comprehensive clinical rating scale for the assessment of scars (Appendix 1 – the Beausang Clinical Scale). The scale includes scar colour, contour, texture, and distortion. Each of these values is given a score of between 1 and 4; increasing values indicating more severe scarring. Whether a scar was matte or shiny is also recorded; the former scores a 1 and the latter scores a 2. An overall assessment from 0 to 10 is also made, with 0 indicating an excellent scar and 10 indicating a poor scar. This score is added to the sum of the individual parameter scores to give an overall score for each scar. Scores range from 5 to 28, with low scores representing clinically well-healed scars. It has been shown to have construct validity[80].

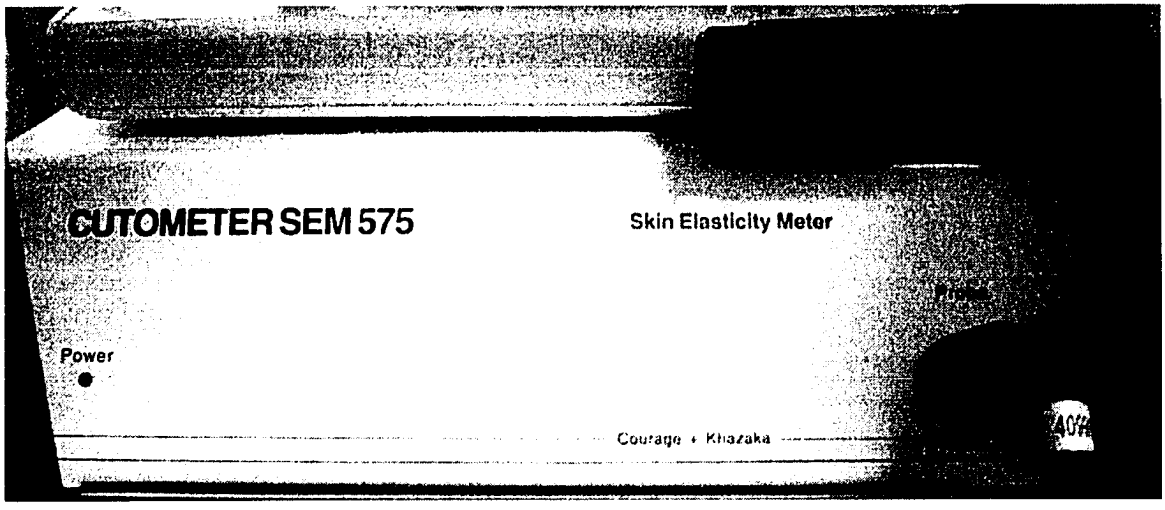
The research assistant responsible for the Beasaung Clinical Scale assessments was trained and supervised by the aforementioned nurse with expertise in wound healing [76, 79]. Assessments were performed on both breasts at the 3 and 6 week follow up appointments.

2.8.2.5 Cutometer skin elasticity meter (SEM) 575®

The Cutometer Skin Elasticity Meter (SEM) 575® (Courage and Khazaka Electronic GmbH, Cologne, Germany) is an automated instrument designed to quantify skin elasticity (Figure 2.5 – Cutometer®). A hand held probe with a 2 mm aperture is placed against the skin. A vacuum load of 500 mbar is applied through the aperture for 1 second, followed by normal pressure for 1 second. The skin is drawn into the aperture of the probe. The depth of penetration of the skin into the probe is determined by an optical measuring system. The parameters recorded were immediate skin distention (Ur) and final skin distention (Uf) in millimeters. The elasticity scores produced by the Cutometer® are a reliable measure of elasticity for both normal skin and scars[81-83]. The research assistant responsible for the Cutometer® assessments was trained and supervised by a the aforementioned nurse with expertise in wound healing [76, 79]. Assessments were performed on both breasts at the 3 and 6 week follow up appointments. For breasts with inverted T incisions, an assessment was done at the T-base and the mid-vertical point on the scar. For patients with either vertical-only or horizontal-only

incisions, assessments were done at the mid-point of the scar. Each scar was measured three times and the average of the three measured values was recorded.

Figure 2.5 – Cutometer Skin Elasticity Meter (SEM) 575® (Courage and Khazaka Electronic GmbH, Cologne, Germany)



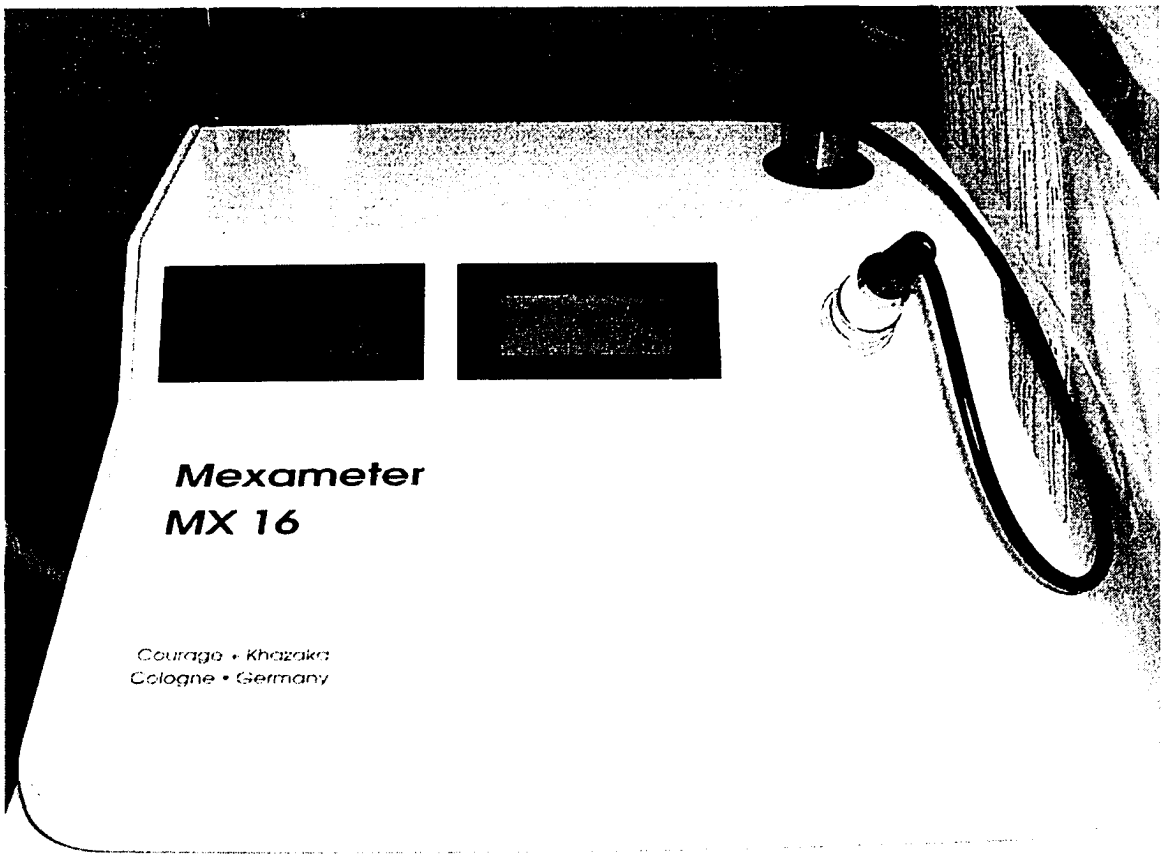
2.8.2.6 Mexameter

The Mexameter MX 16® (Courage+Khazaka Electric GmbH, Cologne Germany) is a narrow-band simple reflectance meter designed to measure skin colour (Figure 2.6 – the Mexameter®). A 5 mm diameter probe emits three wavelengths of light (660 nm, red; 568 nm, green; and 880 nm, infrared). Scores for the level of erythema and melanin are calculated based on the amount of light absorbed by the skin. The erythema index (EI) is defined as: EI = (500/log5) x [log (red-

reflection/green-reflection) + log5]. The melanin index (MI) is defined as: MI = (500/log5) x [log(infrared-reflection/red-reflection) + log5]. The MI and EI scores range from 0-1000 with higher values indicating more melanin and more erythema. The Mexameter® has been shown previously to be a reliable and valid measure of skin colour [84, 85].

The research assistant responsible for the Mexameter® assessments was trained and supervised by the aforementioned nurse with expertise in wound healing [76, 79]. Assessments were performed on both breasts at the 3 and 6 week follow up appointments. For breasts with inverted T incisions, an assessment was done at the T-base and the mid-vertical point of the scar. For patients with either vertical-only or horizontal-only incisions, assessments were done at the mid-point of the scar. Each scar was measured three times and the average of the three measured values was calculated.

Figure 2.6 - Mexameter MX 16® (Courage+Khazaka Electric GmbH, Cologne, Germany)



2.8.2.7 Adverse events

Complications were identified by several mechanisms. At the 1, 3, and 6 week follow-up appointments, patients were questioned by the research assistant about whether they had experienced complications. Hospital operative reports and office

charts were systematically reviewed by the PI to confirm the occurrence of complications identified by patients and to identify any of the other previously defined complications (see section 2.8.2.7).

2.8.2.7.1 Post-operative hematomas

Post-operative hematomas were defined as discomfort and swelling under an incision which was fluctuant on palpation. The diagnosis was confirmed by needle aspiration of sanguinous fluid. Hematomas were graded as mild (< 10 mL), moderate (> 10 mL), and severe (requiring operative drainage). Assessments were made by the attending surgeons. At the 1, 3, and 6 week follow-up appointments, patients were questioned by the research assistant about whether they had experienced these complications.

2.8.2.7.2 Post-operative seromas

Post-operative seromas were defined as discomfort and swelling under an incision which was fluctuant on palpation and did not meet the criteria for diagnosis of hematoma or wound infection. The diagnosis was confirmed by needle aspiration of serous fluid. Seromas were graded as mild (< 10 mL), moderate (> 10 mL), and severe (requiring operative drainage). Assessments were made by the attending surgeons. At the 1, 3, and 6 week follow-up appointments, patients were

questioned by the research assistant about whether they had experienced these complications.

2.8.2.7.3 Post-operative infections

Post-operative infections were defined according to the Centers for Disease Control and Prevention criteria for surgical site infections [86] (Table 2.1). Infections were graded in terms of severity: mild (requiring oral antibiotics), moderate (requiring intravenous antibiotics), and severe (requiring surgical intervention or hospital admission). All assessments were made by the attending surgeons. At the 1, 3, and 6 week follow-up appointments, patients were questioned by the research assistant about whether they had experienced these complications.

Table 2.1 - Centers for Disease Control and Prevention (CDC) guidelines for the diagnosis of surgical site infections (SSIs) [86]

Infection occurs within 30 days after the operation and involves only skin or subcutaneous tissue of the incision, WITH at least ONE of the following:
1. Purulent drainage, with or without laboratory confirmation, from the superficial incision
2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision
3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat *and* superficial incision is deliberately opened by surgeon, *unless* incision is culture negative
4. Diagnosis of the superficial incisional SSI by the surgeon or attending physician

2.9 Data collection – case report forms (CRFs)

Standardized case report forms were used for patient screening and collection of pre-operative, intra-operative and post-operative data (Appendix 1 – CRFs). EPICORE Centre provided these forms.

2.10 Data management

An independent agency (EPICORE Centre) was responsible for data management. All case report forms were either faxed or delivered to EPICORE Centre on completion and were entered into the database. Queries pertaining to any missing or inappropriately completed CRFs were sent to the individual(s) who had collected the information. This included a search for illogical and extreme values. The primary investigator kept a copy of all CRFs until completion of the study. Forms were then sent to EPICORE Centre where they will be kept for 7 years, as per Good Clinical Practice Guidelines.

2.11 Statistical considerations

2.11.1 Sample size

The sample size calculations were based on the primary outcome measure (difference in total drainage over the first 24 hour post-operatively). We assumed a minimum clinically important difference (MCID) of 15 % reduction in drainage output, a standard deviation (SD) of 20 ml, an alpha of 0.05, and a power of 0.95. The MCID of 15% was determined by polling local plastic surgeons. The SD was based on findings of our pilot study (SD = 29, n = 10) and a retrospective review that we conducted on the difference in drainage between breasts after standard

reduction mammoplasty without CAPG (SD 18; n = 19). The power was set at 0.95. Reasons for this level of power included: (1) the agent was already in widespread use, (2) the inclusion of increased number of surgeons and different types of breast reduction techniques was likely to increase the variability in drainage, and (3) we believed that with a negative result, the study was unlikely to be repeated. Based on these, numbers the necessary sample size estimate was 92 patients. To account for a 10 % attrition rate, 102 patients were required.

2.11.2 Statistical analysis

2.11.2.1 Description of the sample

Baseline characteristics of the study population were described in order to describe the patient population. Normally distributed and skewed continuous data was presented using means (standard deviations (SD)) and medians (interquartile ranges (IQRs)), respectively. Categorical data was presented using proportions.

2.11.2.2 Comparison of baseline characteristics

Baseline characteristics of the breasts were compared to determine whether randomization was effective. Treated and control groups were compared with regards to known prognostic factors using the Student's t-test (for continuous

dependent variables) and the Pearson chi-square test (for categorical dependent variables).

2.11.2.3 Comparisons of outcomes

The primary outcome variable (difference in drainage during the first 24 hours post-operatively) was analyzed using the paired-t-test and sign-rank tests. Secondary outcomes of pain, size of open area, RVSS score, Beausang clinical scar score, Cutometer® score, Mexameter® scores, and adverse events were analyzed using paired-t-tests and sign-rank tests.

2.11.2.5 Predictors of increased drainage

Predictors of increased drainage were determined by multiple linear regression. For this analysis, the patient's total drainage from both breasts over the first 24 hours was used as the unit of analysis. The principles of purposeful regression were applied. Clinically important variables and those having p-values less than 0.20 in simple bivariate correlation were used in the full model. These variables were then entered into a full model. Variables were then removed from the model until all predictors had p-values > 0.05. This resultant model was designated the main effects model. Variables in the full model that were not included in the

main effects model were tested for confounding. Confounders were added to the main effects model. The model was tested for all 2^{nd}-order interactions and model diagnostics were performed. All analyses were performed using intention to treat principles.

2.12 Summary of outcome measures

Table 2.2 summarizes the each of outcomes assessed during the trial. Included is the time of the assessment and the assessor. All outcome assessors were blinded to treatment allocation.

Time point	Outcome Assessor	Outcome(s) assessed
Every 8 hrs for the first 24 hrs post-operatively	Trained research nurse(s)	1. Drainage 2. Pain
1 week post-operatively	Trained research assistant supervised by the research nurse	1. Pain 2. Complications
3 weeks post-operatively	Trained research assistant supervised by the research nurse	1. Pain 2. RVSS 3. Beausang scar scale 4. Cutometer® 5. Mexameter® 6. Complications
6 weeks post-operatively	Trained research assistant supervised by the research nurse	1. Pain 2. RVSS 3. Beausang scar scale 4. Cutometer® 5. Mexameter® 6. Complications

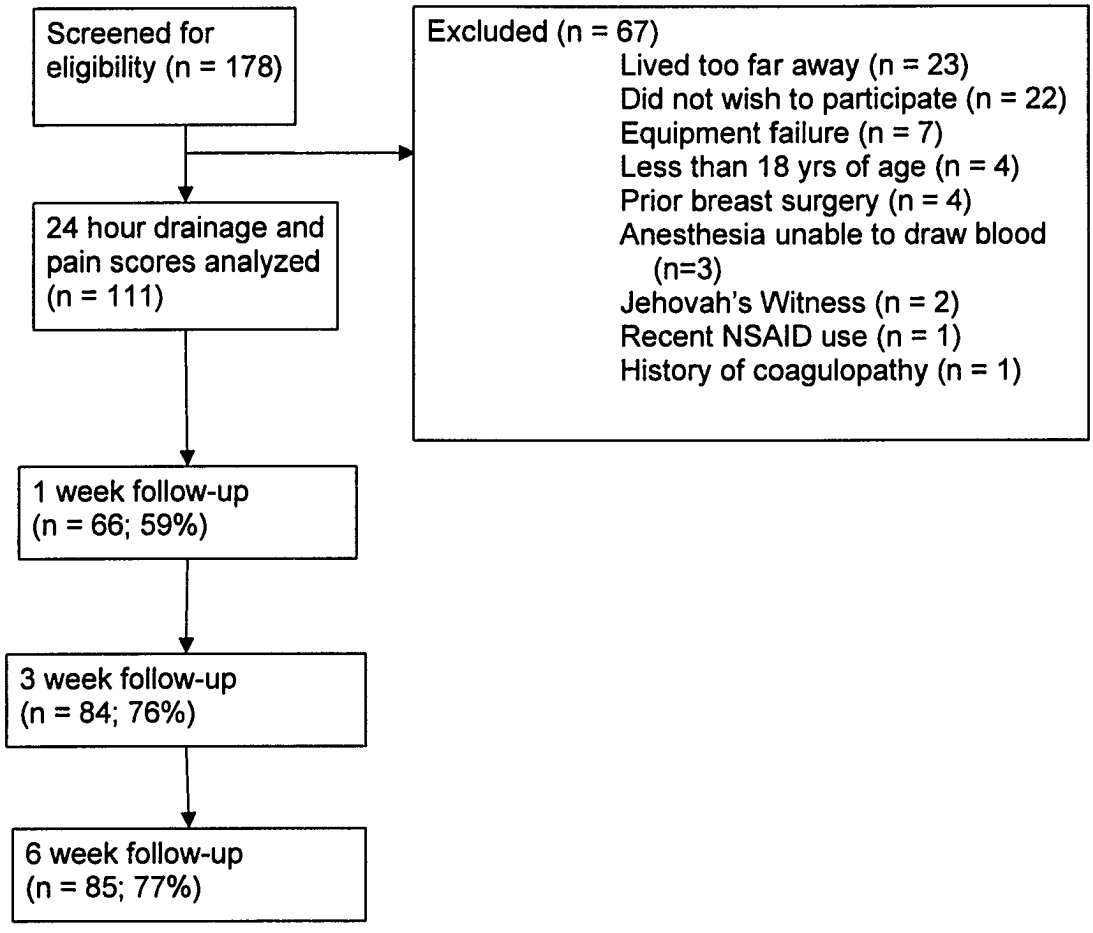
Table 2.2 – Summary of outcome measures

3.0 Results

3.1 Flow of patients through the trial

Figure 3.1 shows the flow of patients through the study. One hundred and seventy-eight patients were screened for eligibility. Sixty-seven were excluded from the trial. The most common reason for exclusion was patients who lived too far from Edmonton to commit to the follow-up appointments. Of note, 7 patients were excluded from the study due to equipment failure. In each case, this was prior to randomization. In one case the citrate was defective. In the remaining 6, there were difficulties closing the centrifuge lid. Twenty-four hour drainage and pain scores were collected for all of the 111 randomized patients. The number of patients returning for their 1, 3, and 6 week follow-ups were 66 (59%), 84 (76%), and 85 (77%) respectively.

Figure 3.1 - Flow of patients through the trial



3.2 Patient characteristics

The characteristics of the women entered into the study are displayed in Table 3.1. Visual inspection of the data with histograms suggested it was not normally distributed. For this reason we used medians and inter-quartile ranges to describe the data. The median age was 39 years, body mass index (BMI) was 31, and

median chest circumference was 38 inches. Seventy-five percent of women had cup sizes DD or greater and 18% were smokers. None of the study participants were diabetic.

Table 3.1 – Preoperative demographics of the patient sample

n = 111	Median	(IQR)
Age (years)	39	(28, 48)
Weight (lbs)*	176	(157, 200)
Height (inches)*	64	(62, 66)
BMI*	31	(27, 34)
Chest circumference (inches)	38	(37, 42)
	Frequency	(%)
Cup Size (n = 111)		
≤ C/D	28	(25%)
≥ DD	83	(75%)
Smoker (Yes)	20	(18%)
Diabetic (Yes)	0	

* n = 110

3.3 Intra-operative characteristics of the study population

Table 3.2 displays the intra-operative data for the women enrolled in the study. Forty-nine percent had pre-operative infiltration with a solution containing local anesthetic and epinephrine. Thirty-one percent had adjuvant liposuction of the breast as part of their surgery. Seventy-one percent had a classic Robin's reduction. A classic Robin's reduction was defined as an inferior pedicle with an inverted T-skin closure. The remaining patients had superiorly pedicle based

reductions. Skin closure in these patients was one of three types: (1) vertical only (2) vertical with a short horizontal or (3) a horizontal only skin closure. For those having infiltration, the median amount was 600 mL. For those having adjuvant liposuction, the median amount was 275 mL. The median amount of tissue removed, from right and left breasts combined, was 976 grams. The median operative time was 100 minutes.

Table 3.2 – Intra-operative data for the study population

n = 111	Frequency	(%)
Pre-operative infiltration	54	(49)
Adjuvant liposuction	34	(31)
Type of reduction		
Classic Robin's reduction	79	(71)
Superior pedicle technique	32	(29)

	Median	(IQR)
Total infiltration (mL)*	600	(80, 1000)
Amount of liposuction (mL)**	275	(188, 925)
Amount of tissue resected (grams)	976	(753, 1378)
Operative time (min)	100	(87, 120)

* n = 51; ** n = 33

3.4 Comparison of baseline characteristics for the treatment and control breasts

Baseline characteristics for the treatment and control breasts are displayed in Table 3.3. With visual inspection of the data there was insufficient evidence to suggest any significant differences between the groups in terms of the amount of

liposuction, infiltration, or tissue resected. This was confirmed with using paired t-tests. There were 101 patients who had a different operator on the treatment and control breasts. Visually checking the data, there was insufficient evidence to suggest a significant difference in the frequency with which the staff surgeon operated on the treatment versus the control side. This was confirmed with a binomial test.

Table 3.3 – Comparison of pre-operative and intra-operative data for the treatment and control breasts

	Treatment Breast	Control Breast
Median amount of infiltration (mL) (n = 51)	300	300
Median amount of liposuction (mL) (n = 33)	150	125
Median amount of tissue resected (grams) (n = 111)	488	477
Primary surgeon was the staff surgeon (frequency) * (n = 101)	53	48

* in 10/111 cases the staff was the primary surgeon on both sides

3.5 Primary outcome: Total drainage in the treatment versus control breast during the first 24 hours post-operatively

The total drainage in the treatment versus control breasts, during the first 24 hours post-operatively, is displayed in Figure 3.2. The box-plot displays the median,

inter-quartile range, outliers, and extreme values for each group. After visually checking the data with a stem and leaf plot, a normal Q-Q plot, and a histogram we did not find sufficient evidence to conclude the distribution was not normally distributed (Figures 3.3, 3.4 and 3.5). Table 3.4 shows the mean for the treatment and control groups were 70.6 and 72.2 mL, respectively. Using a paired t-test we were unable to demonstrate a significant difference in drainage between the two groups (t = -0.424, p = 0.672).

Figure 3.2 – Box-plot of the median amount of drainage over the first 24 hours post-operatively in the treatment and control groups

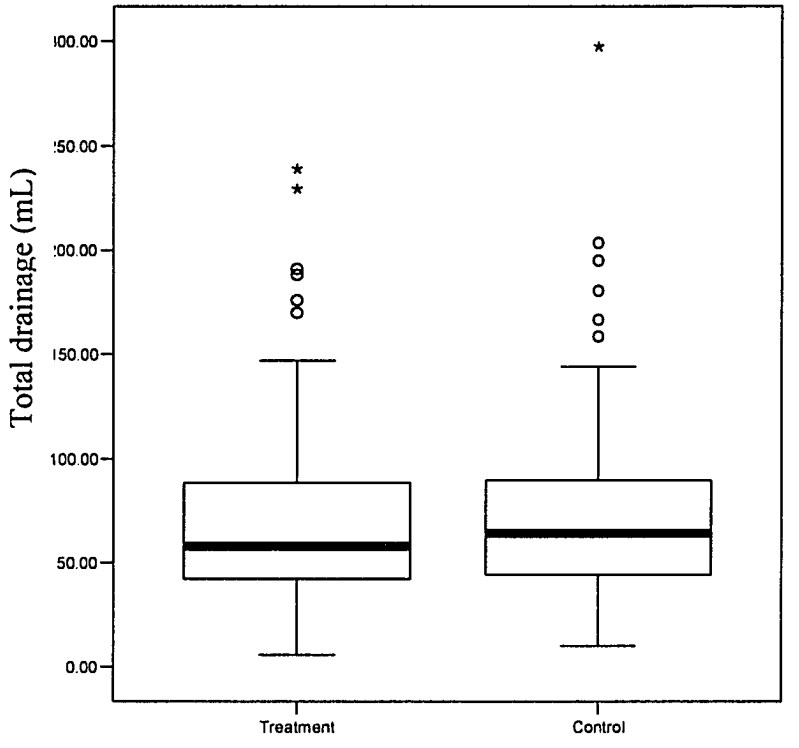


Figure 3.3 - Stem and leaf plot of the difference in total drainage (mL) between the treatment and control breasts during the first 24 hours post-operatively

```
Frequency     Stem &  Leaf

     5.00 Extremes    (=<-2.9)
     1.00       -2 .  6
     3.00       -2 .  023
     3.00       -1 .  568
    10.00       -1 .  0000223444
    16.00       -0 .  5556667777888889
    11.00       -0 .  12222222334
    29.00        0 .  00000000000000000012222224444
    17.00        0 .  55666666667778888
     8.00        1 .  00002344
     4.00        1 .  5677
     2.00        2 .  00
     1.00        2 .  5
     1.00 Extremes    (>=2.9)

Stem width:      1.00
Each leaf:       1 case(s)
```

Figure 3.4 - Normal Q-Q plot of the difference in drainage (mL) between the treatment and control breasts during the first 24 hours post-operatively

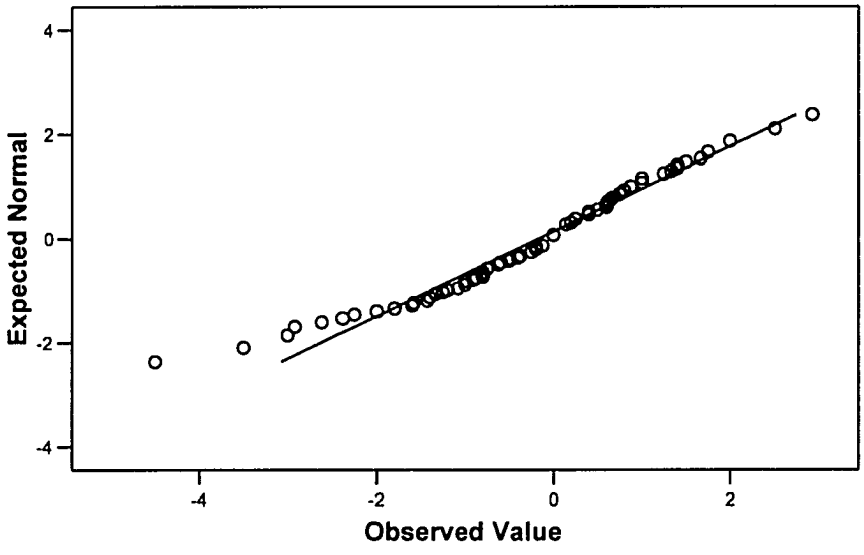


Figure 3.5 - Histogram of the difference in drainage (treatment – control) (mL) between the treatment and control breasts during the first 24 hours post-operatively

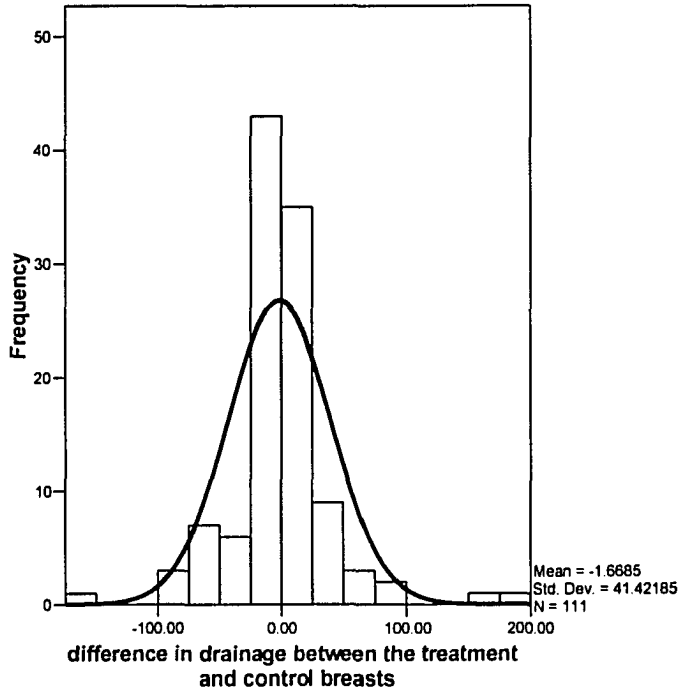


Table 3.4 – Comparison of total drainage during the 24 hours post-operatively for the treatment and control breasts

n = 111	Treatment breast		Control Breast		Difference between the treatment and control breasts		Paired t-test		
	Mean	SD	Mean	SD	Mean	SD	t	p	95% CI
Average drainage over the first 24 hours post-operatively (mL)	70.6	43.1	72.2	45	-1.7	(41)	-0.424	0.672	(-9.5, 6.1)

3.6 Secondary outcomes

3.6.1 Pain in the treatment versus control breasts

The average pain scores of the treatment versus control breasts during the first 24 hours post-operatively are displayed in Figure 3.6. After visually checking the data with a normal Q-Q plot and a histogram, we did not find sufficient evidence to conclude the difference was not normally distributed. A paired t-test was unable to demonstrate a significant difference in pain scores between the treatment (3.3/10) and control (3.5/10) groups ($t = -1.406$, $p = 0.163$) (Table 3.4).

In addition to displaying the average pain scores over the first 24 hours post-operatively, Table 3.5 includes the pain scores at 1, 3, and 6 weeks post-operatively. Visually checking the data with a normal Q-Q plot and a histogram we did not find sufficient evidence to conclude the distributions were not normally distributed. At 1 week, the mean treatment and control pain scores were 2.3 and 2.4, respectively. At 3 weeks, the mean treatment and control pain scores were 2.4 and 2.2, respectively. At 6 weeks the mean treatment and control pain scores were 1.1 and 1.5, respectively. Using paired t-tests, we were unable to find any significant differences in pain between the two groups (1 week: $t = -0.172$, $p = 0.864$; 3 weeks: $t = 0.785$, $p = 0.435$; 6 weeks: $t = -1.754$, $p = 0.083$).

Figure 3.6 – Average pain scores over the first 24 hrs post-operatively on the treatment and control sides

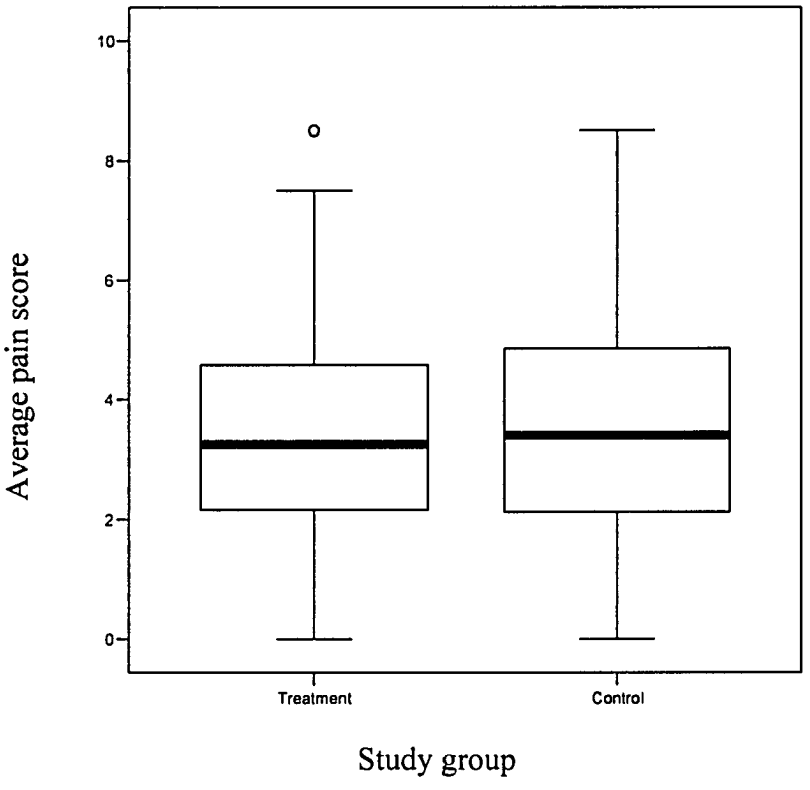


Table 3.5 – Comparison of the pain scores for the treatment and control breasts

	Treatment breast		Control Breast		Difference between the treatment and control breasts		Paired t-test		
	Mean	SD	Mean	SD	Mean	SD	t	p	95% CI
Average pain over the first 24 hours post-operatively (n = 111)	3.3	1.8	3.5	1.9	-0.16	1.2	-1.4	0.163	(-0.39, 0.07)
Pain at 1 week (n = 65)	2.3	1.9	2.4	2.0	-0.05	2.2	-.172	0.864	(-0.58, 0.49)
Pain at 3 weeks (n = 83)	2.4	2.1	2.2	2.0	0.14	1.6	.785	0.435	(-0.21, 0.49)
Pain at 6 weeks (n = 85)	1.1	1.7	1.5	2.4	-0.38	2.0	-1.8	0.083	(-0.82, 0.06)

3.6.2 The size of any remaining open areas in the treatment versus control breasts

The surface area for any remaining open areas on the breasts is displayed in Table 3.6. The size of open areas was checked at 1, 3, and 6 weeks post-operatively. Visually checking the data with a normal Q-Q plot and a histogram, we did found sufficient evidence to conclude the distributions were not normally distributed. This was confirmed with the Kolmogorov-Smirnov test (1 week: K-S = .271 $p < 0.001$; 3 weeks: K-S = .363, $p < 0.001$; 6 weeks: .213, $p < 0.001$). At 1 week the mean open area on the treatment and control breasts were 0.22 and 0.01 mm^2, respectively. Using the Wilcoxon signed-ranked test, we found a significant difference in the size of open areas on the treatment breasts compared to the control breasts ($Z = -2.197$, $p = .028$). Wilcoxon sign-ranked tests were unable to demonstrate any significant differences in the size of open areas between the two groups at 3 and 6 weeks post-operatively (3 weeks: $Z = -0.191$, 0.848; 6 weeks $Z = -1.477$, $p = 0.140$).

Table 3.6 – Comparison of the size of any open areas for the treatment and control breasts

	Treatment breast		Control Breast		Difference between the treatment and control breasts		Wilcoxon signed-rank test	
	Mean	SD	Mean	SD	Mean	SD	Z	p – value
Size of open areas at 1 week (mm^2) (n = 65)	0.22	0.87	0.01	0.03	0.21	0.87	0.345	0.028
Size of open areas at 3 weeks (mm^2) (n = 83)	1.1	5.4	0.60	2.0	0.46	4.9	0.390	0.848
Size of open areas at 6 weeks (mm^2) (n = 84)	0.98	5.6	0.16	0.82	0.82	5.69	-1.477	0.140

3.6.3 The Revised Vancouver Scar Scale (RVSS) summary scores for the treatment versus control breasts

The RVSS summary scores for the treatment versus control breasts are displayed in Table 3.7. Visually checking the data with normal Q-Q plots and histograms we did not find sufficient evidence to conclude the distributions were not normally distributed. Paired t-tests were unable to demonstrate any significant difference in the RVSS summary scores between the two groups (3 weeks: $t = 0$, $p = 1.0$; 6 weeks: $t = .147$, $p = 0.883$).

Table 3.7 – Comparison of the RVSS summary scores for the treatment and control breasts

	Treatment breast		Control Breast		Difference between the treatment and control breasts		Paired t-test		
	Mean	SD	Mean	SD	Mean	SD	t	p	95% CI
RVSS at 3 week (n = 81)	6.7	1.3	6.7	1.3	0	0.5	0	1	(-0.1, 0.1)
RVSS at 6 week (n = 84)	5.6	1.7	5.6	1.4	0.02	1.5	0.147	0.0883	(-0.3, 0.3)

3.6.4 The Beausang summary scores for the treatment versus control breasts

The Beausang summary scores for the treatment versus control breasts are displayed in Table 3.8. Visually checking the data with normal Q-Q plots and histograms we found sufficient evidence to conclude the distributions were not normally distributed. This was confirmed with the Kolmogorov-Smirnov test (3 weeks: K-S = .462, $p < 0.001$; 6 weeks: K-S = .392, $p < 0.001$). Wilcoxon sign-ranked tests were unable to demonstrate any significant difference in the Beausang summary scores between the two groups (3 weeks: Z = -1.587, p = .112; 6 weeks: Z = -.545, p = .586).

Table 3.8 – Comparison of Beausang summary scores for the treatment and control breasts

	Treatment breast		Control Breast		Difference between the treatment and control breasts		Wilcoxon signed-rank test	
	Mean	SD	Mean	SD	Mean	SD	Z	p – value
Beausang Scale at 3 weeks (n = 82)	12.4	2.8	12.1	1.4	.317	2.0	-1.587	0.112
Beausang Scale at 6 weeks (n = 85)	11.9	2.9	11.8	1.4	.13	2.5	-0.545	0.586

3.6.5 Mexameter® scores for the treatment versus control breasts

Mexameter® scores for the treatment versus control breasts are displayed in Tables 3.9 and 3.10. Visually checking the data with normal Q-Q plots and histograms we did not find sufficient evidence to conclude the distributions were not normally distributed. Using paired t-tests, we found the melanin index was significantly lower in the treated breasts compared to the control breasts, for the assessments done at the T-base site (3 weeks: $t = -2.077$, $p = 0.042$; 6 weeks: $t = -2.718$, $p = 0.008$). No other differences were found between the treatment and control breasts for either the melanin index or the erythema index.

Table 3.9 – Comparison of melanin index for the treatment and control breasts

	Treatment breast		Control Breast		Difference between the treatment and control breasts		Paired t-test		
	Mean	SD	Mean	SD	Mean	SD	t	p	95% CI
T-Base site									
Melanin index at the T-base at 3 weeks (n = 69)	440	32	448	44	-8.7	35	-2.077	0.042	(-17, 0.3)
Melanin index at the T-base at 6 weeks (n = 74)	429	33	434	38	-5.3	17	-2.718	0.008	(-9.2, -1.4)
Mid-scar site									
Melanin index at mid-scar at 3 weeks (n = 76)	445	43	445	46	-0.7	19	-0.296	0.768	(-5, 3)
Melanin index at mid-scar at 6 weeks (n = 82)	432	37	433	32	-1.4	14	-0.942	0.349	(-4, 2)

Table 3.10 – Comparison of erythema index for the treatment and control breasts

	Treatment breast		Control Breast		Difference between the treatment and control breasts		Paired t-test		
	Mean	SD	Mean	SD	Mean	SD	t	p	95% CI
T-Base site									
Erythema index at the T-base at 3 weeks (n = 69)	661	30	657	35	4.7	41	0.963	0.339	(-5, 14)
Erythema index at the T-base at 6 weeks (n = 74)	671	23	669	21	1.2	26	0.407	0.685	(-4, 7)
Mid-scar site									
Erythema index at mid-scar at 3 weeks (n = 76)	635	30	636	32	-1	31	-0.288	0.774	(-8, 6)
Erythema index at mid-scar at 6 weeks (n = 82)	655	27	655	29	-0.1	27	-0.043	0.966	(-6, 6)

3.6.6 Cutometer® scores for the treatment versus control breasts

Cutometer® scores for the treatment versus control breasts are displayed in Tables 3.11 – 3.12. Visually checking the data with normal Q-Q plots and histograms we did not find sufficient evidence to conclude the distributions were not normally distributed. Using paired t-tests, we found no differences between the final skin distention (Uf) scores of the treatment and control breasts. Using paired t-tests we found no differences between the immediate skin distention (Ur) scores of the treatment and control breasts.

Table 3.11 – Comparison of the final skin distention (Uf) scores for the treatment and control breasts

	Treatment breast		Control Breast		Difference between the treatment and control breasts		Paired t-test		
	Mean	SD	Mean	SD	Mean	SD	t	p	95% CI
T-Base site									
Uf at the T-base at 3 weeks (n = 63)	0.1647	0.07	0.1599	0.07	0.0048	0.08	0.497	0.621	(-0.0144, 0.0239)
Uf at the T-base at 6 weeks (n = 71)	0.1532	0.06	0.1533	0.06	<0.0001	0.05	-0.015	0.988	(-0.0124, 0.0122)
Mid-scar site									
Uf at mid-scar at 3 weeks (n = 72)	0.1760	0.06	0.1762	0.06	-0.0002	0.06	-0.029	0.997	(-0.01462, 0.01419)
Uf at mid-scar at 6 weeks (n = 80)	0.1736	0.07	0.1731	0.06	0.0005	0.06	0.076	0.940	(-0.0131, 0.0142)

Table 3.12 – Comparison of the immediate skin distention (Ur) scores for the treatment and control breasts

	Treatment breast		Control Breast		Difference between the treatment and control breasts		Paired t-test		
	Mean	SD	Mean	SD	Mean	SD	t	p	95% CI
T-Base site									
Ur at the T-base at 3 weeks (n =63)	0.0962	0.05	0.0882	0.04	0.0080	0.06	1.119	0.268	(-0.0063, 0.0222)
Ur at the T-base at 6 weeks (n =71)	0.0986	0.04	0.0990	0.04	-0.0004	0.05	-0.069	0.945	(-0.0118, 0.0110)
Mid-scar site									
Ur at mid-scar at 3 weeks (n =72)	0.0925	0.04	0.0997	0.07	-0.0072	0.08	-0.800	0.426	(-0.0252, 0.0108)
Ur at mid-scar at 6 weeks (n =80)	0.1181	0.10	0.1069	0.04	0.0112	0.10	0.956	0.342	(-0.0121, 0.0346)

3.6.7 Adverse events for the treatment versus control breasts

The frequency of adverse events for the control and the treatment breasts is displayed in Table 3.13. Visually inspecting the data there were no significant differences between the groups, this was with binomial tests.

Table 3.13 – The frequency of adverse events for the control and the treatment breasts

n = 111	Frequency in the treatment breasts	%	Frequency in the control breasts	%	Binomial test, p-value
Hematomas requiring re-operation	2	2	2	2	1.000
Infections requiring antibiotics	7	6	6	5	1.000
Other complications	5	5	7	6	0.774

* - Fischer's exact test was used as 2 cells (50.0%) have expected count less than 5.

3.7 Construction of a multivariate model to explain drainage during the first 24 hours post-operatively

In order to construct a model to explain the total drainage during the first 24 hours post-operatively we used a purposeful linear regression technique. Simple bivariate correlation analysis was conducted for all known and potentially important independent variables (Table 3.14). Independent variables with a trend towards a significant correlation ($p < .20$) were included in the full multivariate model.

Table 3.14 - Simple bivariate correlations between the patient's total drainage over the first 24 hours and possible predictors

	Pearson correlation	p-value (2-tailed)	N
Total amount of infiltration (mL)	.515	<.001	111
Length of the OR (min)	.163	.087	111
Total amount of tissue removed (grams)	.305	.001	111
Total amount of liposuction (mL)	.480	<.001	111
Reduction other than a classic Robin's	.654	<.001	111
Chest circumference (inches)	.201	.035	110
Positive smoking history	.095	.326	109
Patient's age (years)	.027	.78	111
BMI	.232	.015	110
Cup size $\geq$ DD	.026	.791	110

If in the full model there were independent variables with p-values > 0.05, the variable with the highest p-value was removed. This process was repeated until all independent variables had p-values $\leq$ 0.05. In our analysis this led to the removal of five variables. The independent variables included in the main effects model were type of breast reduction and amount of tissue removed. A check for confounding effects of the removed variables, on 'important' variables which stayed in the model was performed. Confounding was suspected if addition of a potential confounder to the model led to a change in the value of the beta

coefficient of > 15 %. In our main effects model, the variable considered to be 'important' was type of breast reduction. Both the total amount of infiltration and the total amount of liposuction were identified as confounders. Thus our final model contained type of breast reduction, amount of tissue removed, amount of infiltration, and amount of liposuction. We tested all possible first order interactions for variables included in the main effects model. No significant interactions were found.

Once the final model was defined, we conducted model diagnostics (Table 3.15). We found the variance of the residuals increased as the predicted value of the dependent variable increased. This violated the assumption of homoscedasticity. This was corrected with a natural log transformation of the dependent variable (Table 3.16). A histogram of the studentized deleted residuals of the transformed model show they were normally distributed (Figure 3.7).

Table 3.15 - Original model developed by purposeful linear regression analysis to predict wound drainage after breast reduction surgery

Variable	Unstandardized Coefficients		p-value	95% Confidence Interval for Beta
	Beta	Standard Error		
Total tissue (grams)	0.022	0.009	0.016	(0.004, 0.039)
Reduction other than a Classic Robin's	83.057	16.001	<0.001	(51.327, 114.788)
Total infiltration (mL)	0.017	0.020	0.402	(-0.023, 0.056)
Total liposuction (mL)	0.027	0.017	0.107	(-0.006, 0.061)

Dependent variable: Total drainage (mL)
R-squared: 0.485
Durbin-Watson: 1.798

Table 3.16 - Transformed model developed by purposeful linear regression analysis to predict wound drainage after breast reduction surgery

Variable	Unstandardized Coefficients		p-value	95% Confidence Interval for Beta
	Beta	Standard Error		
Total tissue (grams)	0.0001297	0.0000663	0.041	(0.0000052, 0.0002543)
Reduction other than a Classic Robin's	0.5134212	0.1138881	<0.001	(0.288, 0.739)
Total infiltration (mL)	0.0001192	0.0001406	0.398	(-0.0001596, 0.0003981)
Total liposuction (mL)	0.0001639	0.0001197	0.174	(-0.000073, 0.0004013)

Dependent variable: natural log of total drainage (mL)
R-squared: 0.418
Durbin-Watson: 1.722

Tests for multicollinearity were performed on the transformed model. The largest variance inflation factor was less than 10 and the tolerance smallest tolerance value was greater than 0.2. These values do not suggest the model is at risk of bias due to multicollinearity. Five cases had residuals that were ≥ 2 standard deviations, each of these were investigated for errors. By definition, 5% of the residuals will have values ≥ 2 standard deviations. Visual analysis of a scatter plot of the studentized deleted residual against the unstandardized predicted value did not demonstrate that the variance of the residual changed with changes in the predicted values (Figure 3.8). Figure 3.9 is a scatter plot of the studentized deleted residuals against the centered leverage values. There were seven cases with

centered leverage values above the calculated cut-off 0.09. None of these cases had residuals with an absolute value ≥ 2. There are no cases with a Cook's Distance >1 (Figure 3.10). We assessed the standardized DFBETAs for each variable included in the model and no standardized DFBETA value was greater than 1, Figures 3.11 – 3.15.

Model summary statistics included the R-squared value and Durbin-Watson statistic (Tables 3.15 and 3.16). The R-square was 0.418, indicating the model predicted 41.8% of the variability in wound drainage. The Durbin-Watson statistic was close to 2 which indicates the assumption of independent errors is tenable. The final model for drainage is shown in equation 1.

Equation 1 – Final model for total drainage over the first 24 hours post-operatively

$$Y = e^{[(0.000130 * \text{total tissue resected in grams}) + (0.513 * \text{type of reduction}) + (0.000119 * \text{total infiltration in mL}) + (0.000164 * \text{total liposuction in mL})]}$$

Note : Y = total drainage (mL)

Type of reduction is 0 for a Classic Robin's reduction and is 1 for all other types

Figure 3.7 - Histogram of the studentized deleted residuals from the final model

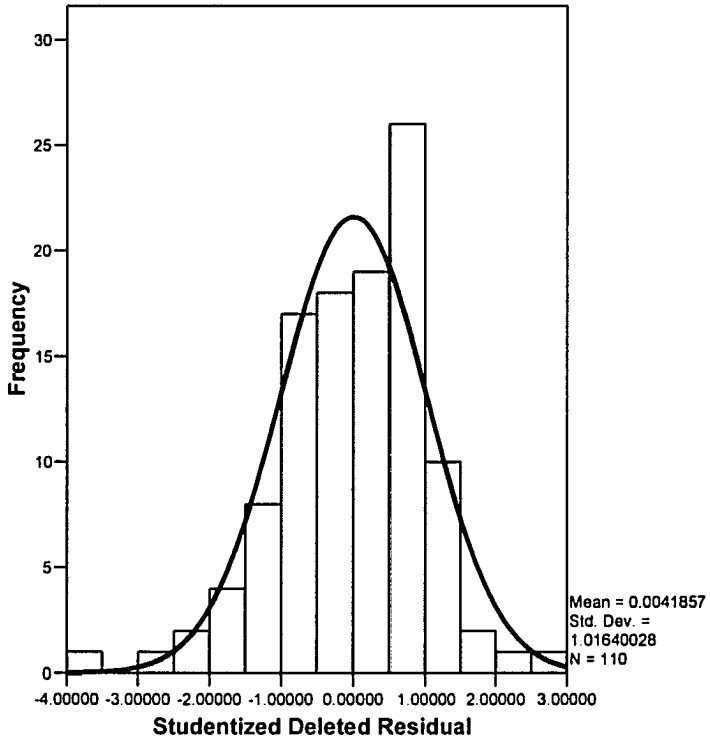


Figure 3.8 - Scatter plot of the studentized deleted residuals against the unstandardized predicted values

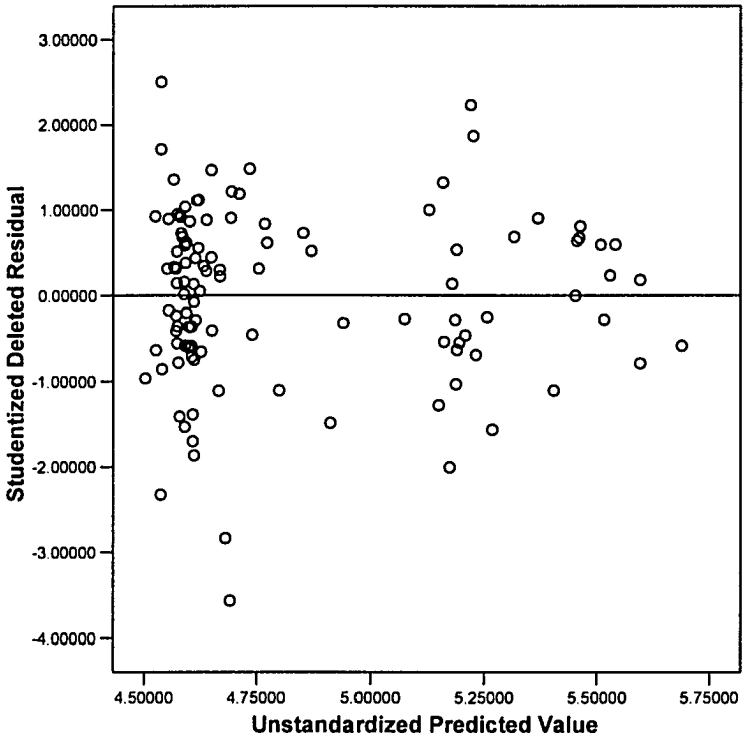


Figure 3.9 - Scatter plot of the studentized deleted residuals against the centered leverage values

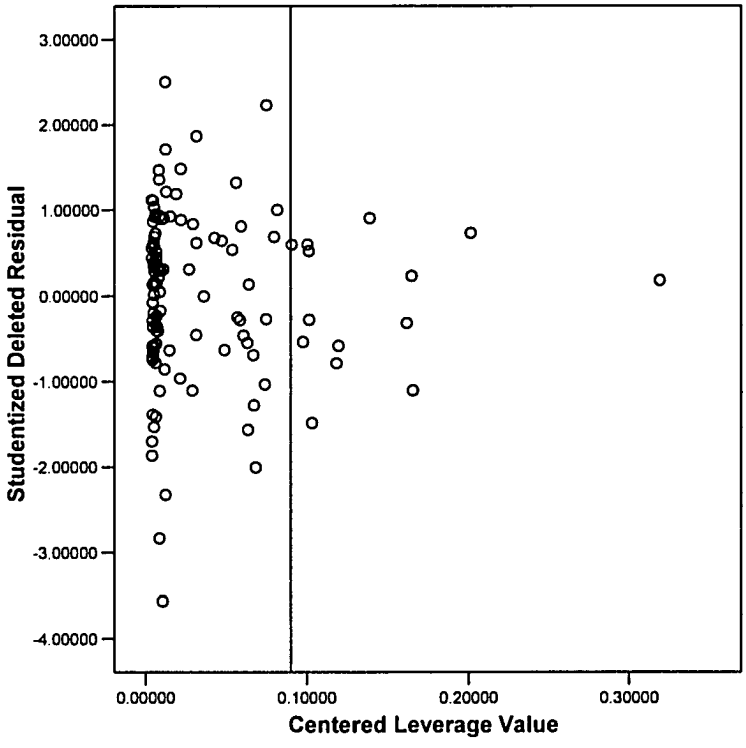
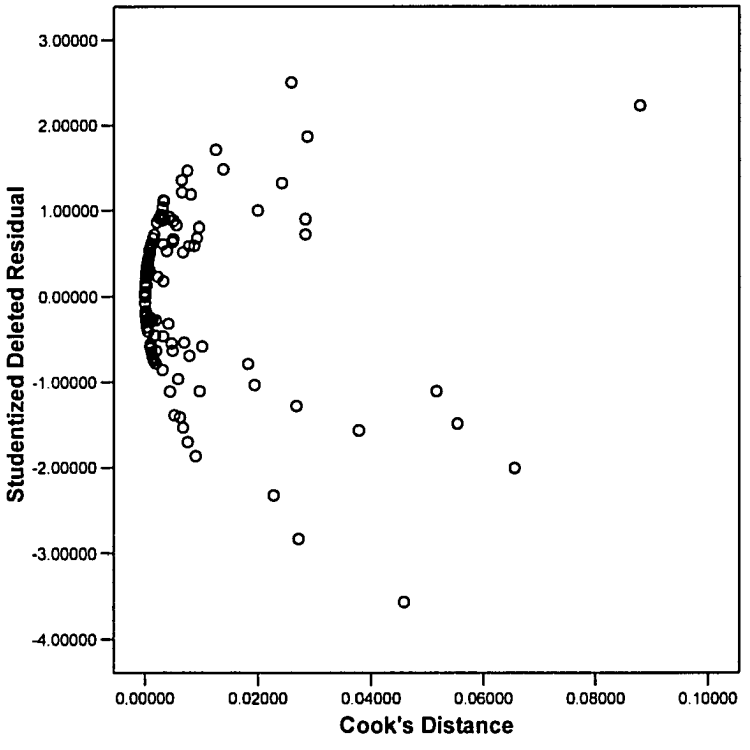
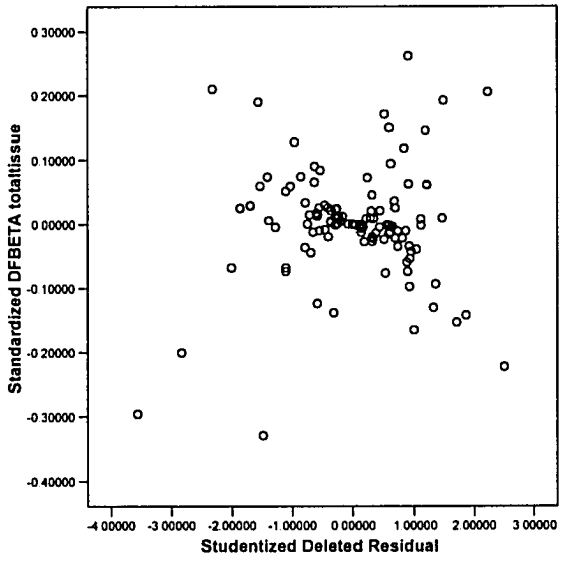
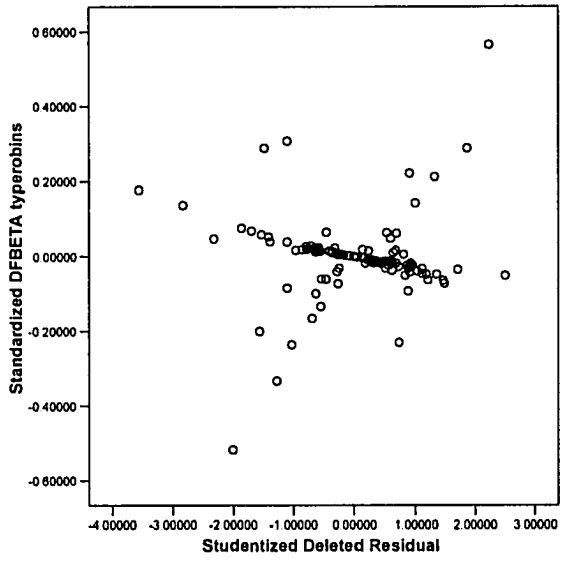


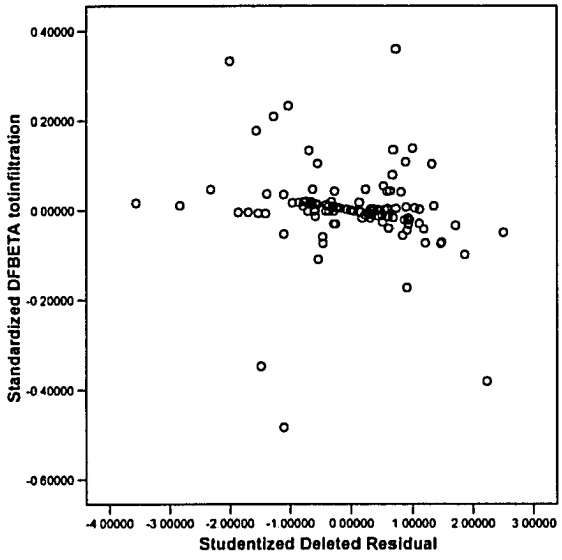
Figure 3.10 - Scatter plot of the studentized deleted residuals against Cook's Distances

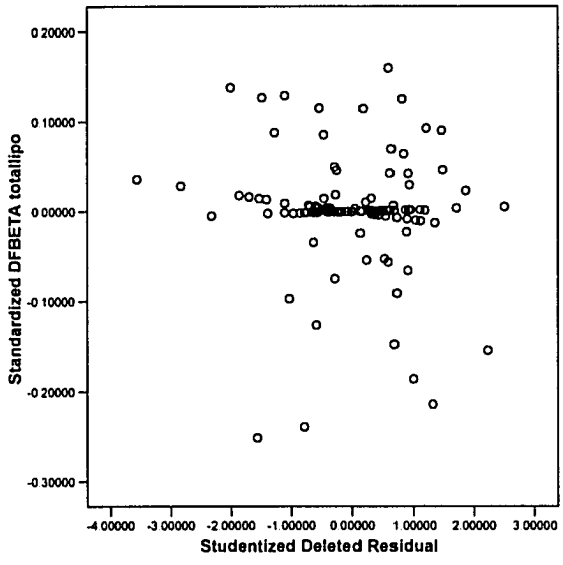


Figures 3.11, 3.12, 3.13, and 3.14 - Scatter plots of the standardized DFBETA for each variable in the model against studentized deleted residuals









4.0 Discussion

4.1 Description of the study population

The patients included in this study are similar to those studied in the most comprehensive BRM study conducted by Collins[87]. He included 291 from 15 different cities across the United States. The patient characteristics reported in his study were nearly identical to our study population. In both studies, the average age was 39 years, the median cup size was DD, and the average amount of tissue resected was similar (976 versus 814 grams). The average BMI in Collin's group was 30, compared to an average BMI of 31 in our study population.

There were some differences between the surgical techniques used in our study and that of Collins. The frequency with which patients had adjuvant liposuction was higher in our study (31% versus 14%) and the amount suctioned was larger (mean of 75 mL versus 139 mL). This is likely because the Collins study was conducted 5 years earlier and the use of adjuvant liposuction has recently increased in popularity[88, 89]. Another difference was the average operating room time. The duration of surgery was shorter in our study population (81 versus 155 minutes). This is likely a reflection of the differences between the American and Canadian health care systems. American surgeons have unlimited operative time and are limited only by the number of patients requiring surgery. Canadian

surgeons have a high patient load and limited operative time. As a result, Canadian surgeons have an incentive to minimize the total operative time.

4.2 Primary outcome

The primary outcome measure was the difference in drainage between the CAPG-treated breasts and the control breasts over the first 24 hours post-operatively. Treated breasts drained less than the controls (70.6 versus 72.2 mL); however, this difference was not statistically significant ($p = 0.672$), nor would a difference of only 1.6mL be clinically significant (we defined our MCID to be a change in drainage of 15% , in this case, 10.5 mL).

4.2.1 Reasons why there was no difference in drainage observed

The reason why the application of CAPG did not decrease the amount of wound drainage is not entirely clear. The application of CAPG to the subcutaneous tissues after BRM may simply be ineffective at decreasing drainage (i.e., the intervention tested does not work). However, there are several alternative explanations that must be considered.

4.2.1.1 Power

A *post hoc* power calculation was conducted to determine the likelihood that the negative result was due to a type II error. The original sample size calculation was designed to achieve a power of 95%. The estimate of the standard deviation (SD) used in the sample size calculation (20 mL) was based on the results of a pilot study and a retrospective chart review. The SD for the average amount of drainage during the first 24 hours post-operatively among the study population was twice as large (41 mL) as our original estimate. The higher observed variability was likely due to the fact that the population used to calculate the sample size estimate included only patients with Classic Robin's type reductions. The inclusion of patients with superior pedicle type reductions into the study increased the variability of the drainage. We conducted a *post hoc* power calculation using the higher SD of 41 mL. It suggested that our study still had 80% power to detect the MCID of a 15% difference in drainage. Since, this is considered an acceptable level of power[90], and it is unlikely that a type II error occurred.

4.2.1.2 Quality of the CAPG produced

It is possible that the application of CAPG was ineffective because it did not contain high levels of active platelets. Previous studies have suggested that the level of activated platelets should be in the range of 700,000 platelets /

microliter[36, 44]. Weibrich compared the level of platelets and growth factors contained in platelet gels prepared using several different systems. He found a significant difference in both platelet number and growth factor concentrations [91-94]. In this study, we employed the Magellan® dual speed separator system. The quality of the CAPG produced by this system has been previously tested by Medtronic Inc. These studies found the final concentration of active platelets produced averaged 1,344,890 platelets / microliter[95]. In addition, there was a significant increase in the concentration of TGF-beta, PDGF, VEGF, and EGF in the CAPG compared to whole blood[95]. Based on these findings, it is likely that the quality of the CAPG produced by in this study was within the desired specifications.

4.2.1.3 Blocked drains

Blocked drains would not explain our finding of no difference, and would have produced a positive bias. A positive bias is a systematic error which leads to an incorrect overestimation of the true effect size. The CAPG may have blocked the drain holes and prevented fluid from exiting the breasts. The CAPG is designed to form a fibrin clot at the site of application. If this clot had formed over the drain holes on the treatment side, the fluid within the breast would not have emptied into the Jackson-Pratt drain. In our pilot study, we assessed the drains for the

possibility of such a blockage. Drains were inspected at the time of removal and no blockages were noted.

4.2.2.3 Interference of clot formation due to the suction drains

It is possible that the use of suction drains after the application of CAPG prevented a stable clot from forming and interfered with the CAPG's capillary-sealing effects. However, the CAPG was applied prior to closure of the breast tissue and the drains were not primed until the skin closure was complete on both sides. This resulted in an average of 30 minutes between CAPG application and priming of the drains. This period should have allowed enough time for the formation of a stable CAPG clot.

Other investigators have demonstrated the effectiveness of tissue sealants in wounds where drains were left in place. Matthews studied the effectiveness of a fibrin sealant in decreasing wound drainage after thyroid surgery[96]. Thirty patients were treated with fibrin sealant and 30 were included in the control group. All patients had suction drains left in place. The drainage in the treatment group was significantly less than in the control group (18 versus 39 mL, $p < 0.0001$). This study suggests that the use of suction drains does not prevent an effective tissue sealant from decreasing drainage.

Finally, the placement of suction drains is considered standard of care when surgeons are concerned about high levels of post-operative wound drainage. It is under these conditions that CAPG might be indicated. To be useful, a tissue sealant must be an effective adjunct to suction drains.

4.2.3 Summary of the primary outcome measure

In summary, there was no difference between the CAPG-treated and control breasts in the amount of wound drainage over the first 24 hours post-operatively. The most likely reason no difference between the groups was observed was because the null hypothesis is correct – that CAPG does not decrease drainage.

4.3 Secondary outcomes

As specified in the study protocol, secondary outcomes were collected in order to assess the effect of CAPG on pain, wound healing, and complication rates. These secondary outcomes included comparisons of pain, the size of open areas, clinical scar assessments with the Beausang and RVSS scores, Cutometer® assessments, Mexameter® assessments, and complication rates between the treatment and control breasts.

4.3.1 Analgesic effects

Using a 0-10 numerical rating scale, pain was assessed a minimum of every eight hours for the first 24 hours post-operatively. In addition, pain was assessed at the 1, 3, and 6 week follow-up visits. We observed no significant difference in pain between the treatment and control breasts. This is in contrast to previous studies which showed a reduction in post-operative pain with the application of platelet gel[23, 30]. Those studies also found a decreased in wound drainage. It is possible that this decrease in pain was a result of the decrease drainage, rather than a direct effect of the platelet gel.

Alternatively, the studies demonstrating an analgesic effect of platelet gel may have been prone to positive biases. In the study by Floryan, there were 27 patients in the treatment group and 23 patients in the control group[30]. Patients were not randomized and no blinding was reported. The mean pain score in the treatment group was lower than that of the control group (6.3/10 versus 3.6/10). No statistical analysis was reported and it is not clear whether this difference was due to chance or bias. The study by Monteleone was a within patient study design[23]. Each patient had two skin graft donor sites and one was treated with platelet gel while the other was treated with bovine thrombin. The authors did not test whether their control (bovine thrombin) may have increased the level of pain at the donor site. Twenty patients were included in the study. At 7, 14, 20, and 30 days post-

operatively there was a statistically significant lower pain score in the treated group. Again, sites were not randomized and no blinding was reported. To avoid the risks of bias patients were randomized and outcome assessors were blinded in our study.

4.3.2 Secondary outcomes of wound healing

In order to assess the effectiveness of CAPG in improving wound healing, patients were seen in follow-up at 1, 3, and 6 weeks post-operatively. At each visit, the size of open areas was measured. At the 3 and 6 week visits, clinical scar assessments were made using the RVSS and the Beausang scar scale. In addition, the Cutometer® and Mexameter® were used to measure scar pliability and colour.

4.3.2.1 The size of open areas

A statistically significant difference in wound healing was found between the treatment and control breasts at the 1 week follow-up visit, but not at the 3 and 6 week follow-ups. At the end of 1 week, treated breasts had an open area of 0.22 mm^2 compared to 0.01 mm^2 in the control group. There are several reasons that this finding is not likely to be of clinical value. Firstly, a difference of 0.21 mm^2 between groups is not considered clinically significant. Secondly, the difference between groups was no longer present at the 3 and 6 week follow-ups. Finally, the

number of patients lost to follow-up at the 1 week visit was 10% higher than at the 3 and 6 week follow-ups. Thus, the data from the 1 week follow-up had a higher risk of bias due to loss to follow-up (assuming the patient lost to follow-up at 1 week had smaller open areas on the treatment side)[90]. Based our findings, it is unlikely that the application of CAPG produces an important difference in the size of open areas.

4.3.2.2 Clinical scar assessment scales

Both the RVSS and the Beausang clinical scar assessment scales were used to assess the effects of CAPG on wound healing. When analyzing the results of the scar scales, we found no difference between the treated and control breasts. Although these measures have shown construct validity, they are at risk of having low intra-observer reliability[97-99]. As such, small differences in scar characteristics between sides are unlikely to be identified using these clinical scar scales. One method of increasing the reliability of clinical scar assessments is it to use multiple observers. However, the employment of multiple research assistants to assess each scar was not feasible. In order to improve our ability to identify small, but potentially important, differences in wound healing, we used several outcomes to measures wound healing. These included the Mexameter® and Cutometer®.

4.3.2.3 Cutometer®

The Cutometer® was used to measure differences in wound pliability. The parameters recorded were immediate skin distention (Ur) and final skin distention (Uf) in millimeters. The elasticity scores produced by the Cutometer ® are a reliable measure of elasticity for both normal skin and scars[81-83]. We did not find a difference between the treatment and control groups for either the Ur or the Uf values. The Cutometer® has been validated in studies using large surface areas, such as burn scars[99].

4.3.2.4 Mexameter®

The Mexameter® was used to assess scar colour. Abnormal scarring has a tendency to produce highly pigmented and erythematous scars[97]. Scores for the level of erythema and melanin are calculated based on the amount of light absorbed by the skin. The melanin index (MI) and erythema index (EI) scores range from 0-1000 with higher values indicating more melanin and more erythema. The Mexameter® has been shown previously to be a reliable and valid measure of skin colour [84, 85]. We found no differences between groups in the erythema index at 3 and 6 weeks. However, we did find a statistically significant difference in the melanin index. Scars at the T-base had a lower melanin index at both 3 weeks (440 versus 448) and 6 weeks (429 versus 434). This finding is

likely explained by the difficulty with wound healing at this site. It has been suggested that the effect size of a treatment will be the largest when the pathology is the most severe[100].

It is unlikely that the difference in the melanin index is clinically significant. Yoshimura conducted a case series where patients with hyperpigmented skin lesion were treated and followed using both clinical examination and Mexameter® assessments[84]. He reported that a decrease in the Melanin index of 35 corresponded to a clinically significant improvement. Guevara followed a series of patients with melsama using both clinical examination and the Mexameter[101]. He found that a mean difference in the melanin index of 20 corresponded to a clinically significant difference. The differences between the treatment and control groups found in our study were between 5 and 9. Based on these studies, it is questionable whether a difference in the melanin index of less than 10 is of clinical significance.

4.3.2.5 Reason why there was no difference in wound healing

The reason why the application of CAPG did not result in improved clinical scar scores or improved Cutometer® scores is unknown. Possible explanations for this include the site of the CAPG application and the type of wounds studied.

The lack of effectiveness of the CAPG in improving wound healing may have been related to the site of application. The primary objective of the trial was to test the effectiveness of CAPG in reducing wound drainage. To provide the highest likelihood of success we applied the CAPG to the subcutaneous tissues prior to wound closure. However, scar formation is a function of the more superficial dermal and epidermal tissues[97]. It is possible that application of the CAPG directly to the skin, rather than the deeper subcutaneous tissues, would have improved its effectiveness.

An alternate reason for the lack of effectiveness of CAPG in improving wound healing may be related to the type of wounds studied. Previous human research suggesting that CAPG may be effective in soft-tissue healing has focused on chronic wounds. Rees conducted a double-blind RCT assessing the effectiveness of platelet derived growth factor in treating chronic pressure ulcers[26]. He demonstrated that treated wounds had a significant decrease in ulcer volume compared to the control group. Margolis conducted a retrospective review of the effectiveness of platelet gel for the treatment of diabetic foot ulcers[102]. She reported that larger, more severe wounds were more likely to benefit from the application of platelet gel. It is possible that the CAPG is ineffective in improving wound healing in simple surgical wounds, such as those assessed in our study.

4.3.3 Complication rates

There were no differences in the complication rates between the treated and control breasts. The number of hematomas, infections, and other complications were virtually identical for the control and treatment sides. Clinically, the most important post-operative complication was the development of a post-operative hematoma needing reoperation. However, this our study was not adequately powered to detect a difference in the rate of hematoma formation. Based on a hematoma rate of 2%, an alpha of 0.05, a MCID of 1% and a power of 80%, we would have required 525 patients to test the effectiveness of CAPG in decreasing hematoma formation[90].

4.3.4 Summary of the secondary outcome measures

In summary, although there was a difference between the CAPG-treated and control breasts in the size of open areas at the 1 week follow-up and the melanin index at the T-base site at the 3 and 6 week follow-ups, these differences were not clinically significant. Taken together, our study data do not support the use of CAPG to improve wound healing.

4.4 Additional analyses

Purposeful linear regression was used to provide insight into the predictors of post-operative drainage. The resultant model identified that the use of a technique other than the Classic Robin's reduction and increased tissue resection as independent predictors of increased wound drainage. In addition, the amount of liposuction and the total amount of fluid infiltration were added to the model. These variables were confounders of the relationship between type of reduction and amount of drainage.

The use of a technique other than the Classic Robin's style reduction likely increased the amount of drainage because of the large dead space created. Other techniques involved a superior pedicle with tissue resected from the central portion of the breast, resulting in a large dead space for fluid collection. Coveney conducted an RCT to assess the effects of obliterating dead space after breast surgery for cancer[103]. Patients were randomized to have dead space obliterated with a quilting suture or receive routine care. Outcomes assessed included the amount of wound drainage and the rate of seroma formation. Patients randomized to the dead space obliteration group had significantly less drainage (272 versus 393, $p < 0.05$) and fewer seromas (25% versus 85%, $p < 0.001$). Therefore, the large dead space created by the superior pedicle techniques in BRM was likely the cause of increased wound drainage.

The association between increased tissue resection and increased wound drainage has also been previously reported in the breast cancer literature. Salmon conducted a prospective observational study designed to identify risk factors for increased drainage and seroma formation[59]. The amount of drainage and the incidence of seroma formation were significantly correlated to the amount of tissue removed.

Both the total amount of liposuction and the total amount of infiltration were added to the final model. These two variables were positive confounders of the relationship between the type of reduction and the amount of post-operative drainage (addition of either variable to the multivariate model resulted in a change of the beta coefficient for type of reduction by > 15%). Both variables are independently associated with increased drainage in the univariate analysis. In addition, they are associated with superior pedicle type reductions. When performing superior pedicle type reductions, surgeons almost always used large amounts of infiltration and were more likely to use liposuction. The multivariate model suggests these confounders do not completely explain the increased drainage seen with superior pedicle techniques.

This study suggests that the use of a superior pedicle technique and large amounts of tissue resection are related to increased post-operative drainage. This finding may explain why superior pedicle techniques have higher reported complication

rates[5]. Surgeons performing these reductions should anticipate higher amounts of wound drainage. In these cases, suctions drains should be used and left in place for an extended period.

4.5 General Limitations

The major limitations of this study were related to the surrogate nature of the primary outcome and the large number of patients lost to follow-up. Poor reliability of the secondary outcome assessments was not a concern. These were collected by a research assistant who was trained and supervised by the research nurse. Both the Cutometer® and Mexameter® are automated, easy to use instruments.

4.5.1 Drainage as a surrogate for hematoma and seroma formation

One of the major limitations of this study was the use of drainage as a surrogate marker for the more clinically meaningful outcomes of seroma and hematoma. It is well established that seromas and hematomas can lead to increased patient morbidity. They can cause increased pain, infection, poor wound healing, and the need for secondary surgical procedures [6, 7]. In both clinical practice and the scientific literature, post-operative drainage is a well accepted marker for these complications[59-61, 104].

It is common practice for clinicians to use operative drainage as an indicator of post-operative complications[61]. Patients with high amounts of sanguinous drainage are routinely taken back to the operating room for exploration and possible treatment of a hematoma. In addition, patients generally have their drains left in place until the amount of drainage over a 24 hour period drops below a critical cut-off point. Once drainage is below a critical level, surgeons feel the risk of hematoma is low.

Observational studies have shown a link between increased post-operative drainage and the rates of seroma formation. Salmon conducted a prospective study demonstrating a significant correlation between the incidence of post-operative seromas, the duration of suction drainage and the amount of fluid drained[59].

Randomized controlled trials (RCT) assessing other types of interventions designed to reduce wound drainage have shown a decrease in the rates of seroma formation. Kopelman conducted a RCT to assess the effectiveness of drains for the reduction of seroma formation after axillary dissection in breast carcinoma patients[61]. Forty-two patients were randomized to have their drains removed on post-operative day three. Forty-eight patients were randomized to have their drains removed after the drainage was less than 35 mL in 24 hours. He found that early drain removal was associated with a higher incidence of seromas (21% versus 4%, $p = 0.02$). The author identified a strong relationship between the amount of post-

operative drainage and the risk of seroma formation. Of the patients who had their drains removed after the third post-operative day, those who developed seromas had a higher mean drainage (326 versus 200 mL, $p < 0.0001$).

Similar results were independently reproduced by Dalberg[60]. He conducted a RCT to assess the effectiveness of drains for the reduction of seroma formation after axillary dissection in breast carcinoma patients. Ninety-nine patients were randomized to have their drains removed either on the first post-operative day. Another 99 patients were randomized to have their drains removed after the drainage was less than 40 mL in 24 hours. He found that early drain removal was associated with a higher incidence of seromas (48% versus 22%, $p < 0.001$).

Jain conducted an RCT to assess the effectiveness of fibrin glue to reduce of the amount of drainage, the incidence of seroma formation, and the average volume of seromas after breast surgery in breast carcinoma patients[12]. Twenty-nine patients were randomized to receive fibrin glue and 29 patients were randomized to the control group. Patients treated with fibrin glue had statistically significant reductions in the average volume of any resultant seromas (165 mL versus 300 mL, $p < 0.05$).

Finally, the use of hematomas and seromas as the primary outcome for this study would not have been feasible. We found that the combined incidence of seromas

and hematomas needing operative intervention to be 2%. As mentioned previously, we would have required 525 patients in order to use these as our primary outcome[90].

4.5.2 Losses to follow-up

The number of patients lost to follow-up was higher than anticipated. One hundred and eleven patients were enrolled and randomized in this study. Data regarding drainage and pain over the first 24 hours post-operatively (the primary outcome measure) were collected on all patients. However, data were collected for only 60% (66/111), 77% (85/111), and 76% (84/111) of patients at the 1, 3, and 6 week follow-ups, respectively. We had anticipated a smaller loss to follow-up of 10%. The higher than expected loss to follow-up was due to several factors.

4.5.3 Reasons for losses to follow-up

Firstly, many of the patients involved in the study did not live in Edmonton. Some patients drove several hours to the University Hospital for their appointments. All patients were made aware of this requirement prior to enrollment. However, at the time of recruitment, patients might not have realized the difficulty associated with winter driving conditions in Northern Alberta. Other patients might not have felt well enough or might not have been able to arrange for transportation soon after

surgery. This was reflected in the largest loss to follow-up, seen at the 1 week time point.

Secondly, several patients were scheduled for follow-up visits during the Christmas and New Years holiday period. Many patients were away on vacation or unable to attend their scheduled visits due to family commitments. Such patients were brought in for follow-up visits prior to or after the holidays. However, for many patients, this meant they had missed either their 1 or 3 week follow-up.

Thirdly, the efficiency of the research assistants in collecting the secondary outcomes was inadequately monitored. Some research assistants lost patient data and it is unclear how this occurred. Ongoing data monitoring may have reduced these errors. The primary research assistant, on more than one occasion, cancelled an entire day of follow-up appointments. In these situations, patients were given very little or no notice of the change. This made it difficult to rebook appointments and likely resulted in some patients being unwilling to continue participation. Two additional staff were hired in order to help arrange follow-up appointments. Efforts were made to hire and train a new research assistant to obtain the secondary outcomes. However, due to the rapid nature with which patients were being recruited and the short time frame of the trial, a significant amount of data was compromised.

4.5.4 Impact of losses to follow-up on study validity

Although the number of patients lost to follow-up was higher than anticipated, our main findings remain valid. For the primary outcome of wound drainage and the secondary outcome of pain in the first 24 hours, there were no losses to follow-up. Rates of hematomas requiring re-operation were confirmed using a computerized patient tracking system. Any patient having had an operative procedure by a plastic surgeon in Northern Alberta would have produced a record in this database. Thus, there were no losses to follow-up for this secondary endpoint.

For the outcomes of pain, clinical scar assessment, Cutometer®, and Mexameter® scores pairing of the study design reduced the potential for bias that could have resulted from losses to follow-up. Because all patients received the CAPG, the losses to follow-up were not related to exposure. For patients who did not return for follow-up, both the control and the treatment breasts were excluded from the analysis. It is possible that loss to follow-up was related to the patient's response to the CAPG. However, this is unlikely for two reasons. Firstly, the patient demographics of those who did not return for follow-up did not differ significantly from those who did. Secondly, any patient who did not return for follow-up was contacted by telephone and asked why they did not return. None of these patients reported a significant difference between breasts as the reason.

It is unlikely that the losses to follow-up led to false negative results because of an

inadequate sample size. *Post hoc* power calculations were conducted to determine the likelihood that the neutral results were due to type II errors. For the melanin index and erythema indices we used a SD of 30, a MCID of 10 and a sample size of 85, to calculate that our study had greater than 80% power (Z-beta = 1.12) in the melanin index. For the Uf and Ur values we used a SD of 0.06, a MCID of 20% and a sample size of 85, to calculate that our study had greater than 80% power (Z-beta = 2.96) for pliability. Theses are considered acceptable levels of power[90], therefore it is unlikely that a type II error occurred.

In summary, we had 100% follow-up for the primary outcome and higher than expected losses to follow-up for some of the secondary outcomes. These losses were attributed mainly to the harsh winter driving conditions, the distances patients were required to travel, and the need for more supervision of the research assistants. However, we do not believe these losses to follow-up adversely affected the study validity.

4.6 Implications for future research

Data from this trial has led to the development of several future research questions.

4.6.1 Implications for the use of CAPG as a tissue sealant

In its current usage, CAPG is unlikely to be effective in reducing post-operative wound drainage after BRM. Our study was appropriately powered and is the only randomized, controlled, evaluator-blinded trial assessing the effectiveness of CAPG in reducing wound drainage. One of the major concerns with the trial was the possibility that suction drains interfered with the effectiveness of the CAPG. We would suggest an evaluator-blinded RCT where the patients did not receive drains at the wound site. Other trials using wound drainage as a primary outcome have measured this using ultrasound rather than drain outputs[12].

4.6.2 Implications for the use of CAPG to improve wound healing

Future trials assessing the effectiveness of CAPG to improve wound healing may consider focusing on either chronic wounds or surgical wounds at high risk for wound healing problems. As previously mentioned, Margolis noted that platelet gels were more likely to be effective in treating larger, more chronic wounds[102]. The clinical scar assessments of patients in this study demonstrated that very few patients developed problematic wounds. Potential risk factors for abnormal wound healing were collected in our trial. This information may help to identify high risk patients for future CAPG wound healing studies.

4.6.3 Studies needed for interpretation of Mexameter® and Cutometer® scores

Future studies are needed to assist in the interpretation of Mexameter® and Cutometer® scores. These instruments are reliable, valid, and are sensitive to change. However, there is inadequate information to allow interpretation of these results in a clinically meaningful way. Future wound healing studies should assess Mexameter® and Cutometer® scores in comparison to well established outcome measures such as photographic analyses and clinical rating scales. This would allow investigators to determine the MCID for these automated tools.

4.6.4 Implications for surgeons performing superior pedicle type reduction

Our data suggest that patients undergoing superior pedicle type reduction may have higher rates of complication due to the increased dead space created by the surgical dissection. Recent studies have suggested that the use of superior pedicle techniques is increasing. The higher complication rates associated with this technique have previously been attributed to the difficult skin closure and steep learning curve. The large dead space created by the dissection has not been addressed. Surgeons may consider addressing this by using non-absorbable quilting suture to obliterate this dead space. No studies to date have examined this.

4.6.5 Implications for future surgical research

Our findings highlight the importance of conducting randomized controlled, evaluator-blinded studies for the evaluation of surgical therapies. Many surgical therapies are marketed and utilized, with little or no supporting clinical evidence. Our literature review found many reports promoting the use of platelet gels, however these studies did not have appropriate randomization, concealment of treatment allocation, blinding of outcome assessment, or handling of patient attrition in the analysis. Moher conducted a systematic review comparing treatment effect size in trials with adequate versus inadequate treatment allocation[105]. He demonstrated inadequate concealment was associated with exaggeration of treatment effects by around 60%. Schulz conducted a systematic review of 250 trials to assess the effect of double-blinding[106]. He demonstrated that a lack of double-blinding was, on average, associated with an exaggerated treatment effect. Sigurdson assessed the the quality of clinical research in plastic surgery by conducting a review of the seven most popular plastic surgery journals. He reviewed 25,963 articles and found only 145 randomized control trials. In addition, he demonstrated that plastic surgery trials, on average, were of low quality, putting them at risk for biased results[107]. The results of our randomized controlled evaluator-blinded study differed from that of non-randomized unblinded studies assessing the effectiveness of platelet gel. We suggest surgeons

should conduct research in order to obtain the highest levels of evidence for clinical decision making.

4.7 Conclusions

This study does not support the application of CAPG to the subcutaneous tissues during BRM for reduction in wound drainage, reduction in post-operative pain, or improvements in wound healing. The problem of seromas and hematomas after surgery continues to be a significant source of morbidity. Other forms of currently available tissue sealants may improve wound healing, however they are associated with safety concerns. Researchers should use evaluator-blinded RCTs to evaluate alternate therapies for reducing wound drainage.

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6.0 Appendix 1

Contact Information

Platelet Rich Plasma in Breast Reduction Surgery
(PRP Breast Study)

Study ID: [ ][ ][ ] - [ ][ ][ ][ ] **Patient Initials:** [ ][ ][ ]
Surgeon Number — Patient Number — F M L

Affix Patient Hospital ID Label

Telephone Number(s): Home ( ) _____ - _____
Area code

Work ( ) _____ - _____
Area code

Cell ( ) _____ - _____
Area code

Preferred Contact Time: A.M. _____ P.M. _____

Contact during 24-hour Post-operative Period:

Name of Caretaker: _____ _____
Last Name First Name

Address: _____

Telephone: ( ) _____ - _____
Area code

Secondary Contact Person:
(Close family member or friend not living with the patient)

Name: _____ _____
Last Name First Name

Relationship to Patient: _____

Telephone: ( ) _____ - _____
Area code

Notes: _____

_____

PRP Breast Contact Information.doc 27 July 2004

***DO NOT FAX* - this form to the EPICORE Centre**

7.0 Appendix 2