Abstract

The utilisation of liposuction derived autologous fat tissue in cosmetic and reconstructive surgery is increasingly common. There is no standardized method for concentrating liposuction derived fat for re-injection, but a number of approaches exist, many of which require multiple manipulations of the fat tissue prior to injection. The objective of this study was to develop a device which is capable of concentrating the fat product for cosmetic injection without multiple tissue processing steps.

Fat tissue was harvested from pig hind quarters using a conventional liposuction procedure. The resultant fat/saline mixture was then introduced into the new device for concentration. The new device is a membrane controlled superadsorber technology based upon a device developed for the concentration of blood and used in the cardiovascular field. Samples of the fat product were analysed after processing for % fat content and some gross assessment of the morphology of the fat tissue. The effect of exposure/processing time on the level of concentration achieved was also studies with processing time of 30 and 60 minutes being employed in discrete arms of the study.

The results of this investigation confirmed that the membrane controlled superadsorber concentration technology was capable of concentrating fat derived from liposuction of pig hind quarters. A significant quantity of fat/liquid product was harvested from the liposuction procedure, in excess of 1 liter in all experiments. The input concentration of fat in this product was however very low (<12%) and this impacted upon the ability of the device to concentrate to suitable levels. In the first iteration of the device concentration levels of between 29 -32% were achieved. This was considered to be too low for clinical applications and a second design phase was carried out in which a new device was produced with a larger superadsorber chamber capacity (210.9ml v 89.4ml). This new device was associated with

much higher levels of concentration, taking the input concentration from around 10% to 79.14% over 60 minutes. Critically concentration levels of around 74% were achieved after only 30 minutes of processing. Analysis of the fat product after concentration demonstrated that there was a large population of intact adipocytes present

The results of the present study indicate that the new technology is capable of achieving clinically acceptable levels of fat concentration using liposuction derived fatty preparations. The device performs this function without the need for any external procedures or steps, representing an essentially one-stop approach, producing injectable fat preparation from a low concentration input material. The concentrated fat contains a high population of intact adipocytes. Overall this device represents a new integrated, passive approach to fat concentration for plastic and reconstructive interventions, producing an injectable fat product within the syringe.

Acknowledgement

I would like to acknowledge the undeterred faith shown by my supervisor Professor Terry Gourlay. He always guided me whenever I wavered from the path, and encouraged me with patience throughout all aspects of this project.

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Abbreviation

PRP	Platelet-rich plasma
VADS	Volume of adsorption chamber
ePPTE	Polyterafluoroethylene
AFT	Autologous fat transplantation
AFG	Autologous fat graft
AFJ	Autologous fat injection
HemoSep	Haemoconcentrator
PVC	Polyvinyl chloride
BCS	Breast conserving operation
PCV	Packed cell volume
RBC	Red blood cell
WBC	White blood cell
PLA	Plastocyte
OROS	Oil red O stain
AD	AlloDerm

Content

Chapter 1- Introduction

1.1 History of autologous fat transfer

In 1893 the German surgeon Neuber introduced an innovation in the field of cosmetic surgery that was to gain more and more ground and eventually become the paradigm of a typical 21st century restructuring cosmetic operation - autologous fat transplantation used to augment tissue or fill body and skin defects (Neuber, 1893). Neuber reported that he restrictive the facial depression caused by bone tuberculosis with fat from taken from the upper arm (Neuber, 1893; Robert, 2010). Later, Czery (Czery, 1895) used an adipose tissue to reconstruct a benign breast mass, however the transplanted breast developed undesired sequelae in the form of smaller volume and darker colour than the healthy breast. Lexer (1910) suggested that transplantation of larger tissue mass normally associate with better results. In 1911, further progress in this field was made when the first injection of autologous fat tissue by a syringe through a needle was used to fill up a post-rhinoplasty deformity (Burning 1911; Burning1914). Tuffier (1911) reported that to treat pulmonary disease he put fat in to the extrapleural area. Pathology confirmed that fibrous tissue replaced and resorbed most of the implanted fat tissue after 4 months. (Peer, 1950; Peer 1956) found that larger fat implant > 4cm, was able to efficiently prevent bulk loss than various small grafts. Meanwhile, he stated that the fat graft lost more than 2/5 of original weight and corresponding volume after one year or more following the transplant. However, the innovations in the approaches to performing cosmetic surgery had to be paralled by developments in medical technology. A major step in the field took place in 1974, when liposuction surgery was revived by Fischer (Fischer 1975, Fischer 1976, Fischer

1997). He used a rotating electrical device to aspirate fat through a cannula from small incisions (Fischer, 1997).

Since the advent of the advanced medical technology designed for fat harvesting, liposuctioned fat taken from the body for filling defects and augmentation have assumed an increasingly pivotal role in reconstructive surgery. In 1982, Bircoll was the first to use the liposucted autologous fat for filling defects (Bircoll, 1982). Johnson (1987) described the use of autologous fat injection for contouring defects of face, lateral thighs, buttocks (Paul, et al., 2004), breasts as well as nose (Lee, et al., 2008). Krulig suggested the use of 1987, needles and syringes were used to ensure the sterility of the fatty tissue (Krulig, 1987). The early success in this field confirmed that fat could not just be 'aspirated' or extracted to reduce the amount of adipose tissue in a certain body part, more importantly it can be 'inserted' to correct deformations or smooth out skin imperfections. Every year there are approximately 450,000 liposuction operations performed in the UK by members of the British Association of Aesthetic Plastic Surgeons (Brook, 2011).

1.2 Fat harvesting

Questions remain to be answered in relation to the durability of fat transplant from one area of the body to another, and with regard to the long term benefits of liposuction used to reduce regional body fat. The former is in some instances a key cosmetic procedure following major surgery, but the latter in somewhat more cosmetic than reconstructive.

Fischers and his son introduced one of the most commonly performed liposuction surgery process in Rome in 1976 (Flynn and Narins, 1999; Troilius, 1999; Fodor and Watson, 1998). Since then, this technology has undergone a number of improvements including the "wet technique" inventd by Illouz in which the injection of hypotonic saline and hyaluronic acid subcutaneous and Fournier's "criss-cross" technique. Importantly, in 1987 an U.S A dermatologist Jeffrey Kleim reported his "tumescent" technique, which comprise dilute lidocaine and epinephrine solution to infiltrate the subcutaneous area without additional intravenous or intramuscular analgesics (Klein, 1988). The advanced technique possess several virtues that exceeded the previous method, embracing effectively prevention of blood loss and post operative transfusion , elimination of general anesthesia and reduction of patients recovery time.

The safety of liposuction remains controversial. Despite the highly invasive nature of the liposuction procedures, complications rates are relatively low at about 1% (Hanke, et al, 2004; Housman et al, 2002). Most recently there has been a developing interest in ultrasonic liposuction in which fat is mobilised in the tissue using ultrasound delivered either internal or external. This technique reduces the invasive nature of the liposuction procedure considerably, enhancing the patients experience and overall efficiency. Further research continues in this field, but ultrasonic liposuction may be the procedure of choice in coming years.

The complications associated with conventional fat repositioning has led to the investigation of alternative approaches to tissue sculpting, including the use of alloplastic materials (Maly, et al, 2004; Schmidt-Westhausen, et al, 2004; Lombardi,

2004). Alloplastic materials were utilised from the early 1900s and include injectable forms, such as polyacrylamide gel, silicone, collagen, and allogenetic dermis, for example AlloDerm, Branchburg, as well as solid implants: expanded polyterafluoroethylene (ePPTE), Gore-Tex.

Alloplastic materials, although resulting in good cosmetic outcomes are associated with some problems, including foreign body reactions, warping of the material as well as fatigue. Despite of the fact that injectable silicone was widely used in recent years, its use was associated with serious morbidity and its use was superseded by hyaluronic acid and collagen owing to the safety of these materials (Jordan, 2003; Fagien& Elson, 2001; Durati, et al, 1998).

The ideal alloplastic material filler must have consistent and reproducible results, be easy to handle and lack toxicity. In addition, they must possess a tissue-like nature. Hitherto, there is no ideal artificial material available which meets this need. In contrast, autologous fat meets all of the desirable criteria for an ideal contour material for soft-tissue argumentation. Furthermore, Autologous fat transplant is "autologous", nature tissue and is not subject to rejection process which complicate the use of foreign materials (Mark, et al, 2001; Kaufman, 2006). In addition, adipose tissue contains mesenchymal stem cells (MSCs) which has been identified with similar characteristics to that produced from bone marrow and may offer some degree of tissue regeneration (Zuk, et al, 2001; Award, et al, 2003; Fraser, et al, 2006; Code-Green, et al, 2009).

Steam cell proliferation ability of adipose graft is one of the most important factors for fat autograft survival and retention in body. Tissue proliferation relies on a number of essential factors such as nutrient support, signalling of diverse factors and blood supply (Hemmrich, et al, 2005a; Hemmrich, et al, 2005b; Hyoungshin, 2008). It is believed that increased cell proliferation and differentiation would enhance the adipose autograft survival and long term results (Hemmrich, et al, 2005).

Fat in the body is divided into three layers: superficial, intermediate and deep (Rohrich, 1998; Berry & Davies, 2010) (Figure 1.1). It is important that physicians are aware of the structures when considering liposuction as intervention at the wrong level may result in contour irregularities as well as possible skin injury. In general, the deep and intermediate layers can be safely suctioned (Figure1.2) and the superficial layer should remain undisturbed (Rohrich, 1998).

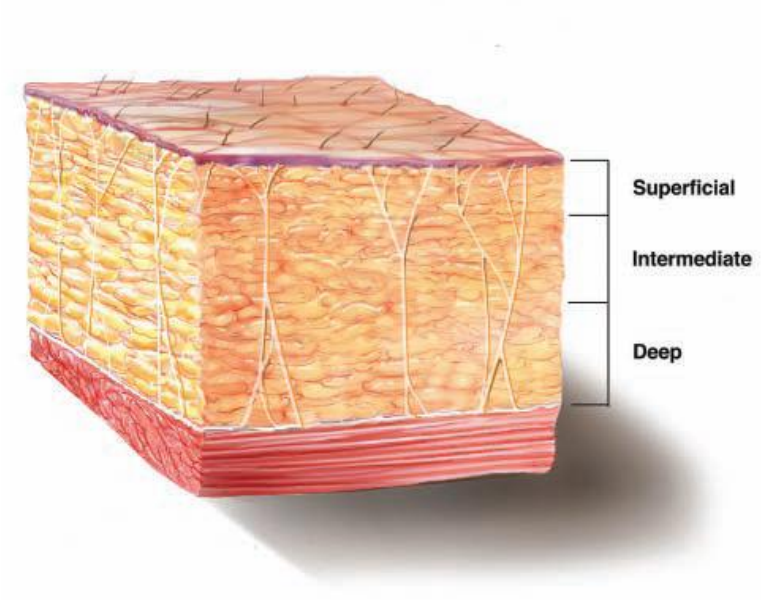


Figure 1.1 Surgical layers of fat: superficial, intermediate and deep. Taken from Carlo, G and Marzia,S (1995).

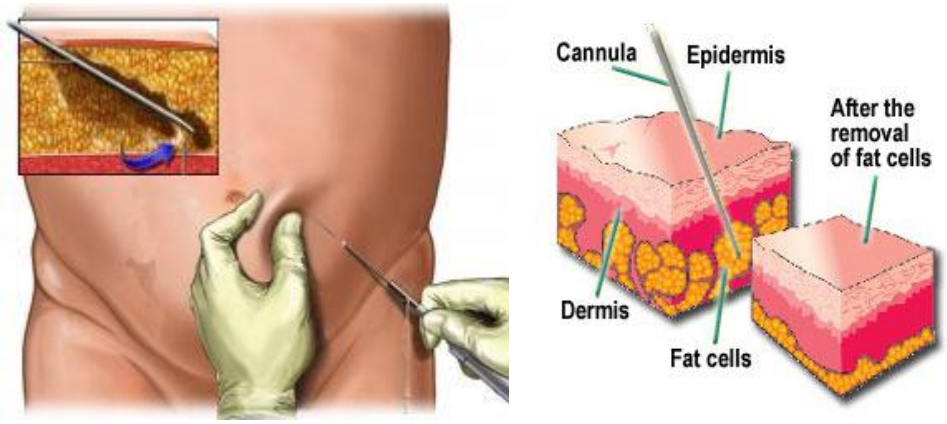


Figure 1.2 Safely suctioning the deep and intermediate layers and the structure of the layers after liposuction. Taken from health all refer com/health/fat-removal- by suctioning-fat -layer-skin.html.

Liposuction is generally employed to reduce the fat level in obese patients or patients who require cosmetic contouring through fat reduction. There are other applications such as fat tissue repositioning. Liposuction for cosmetic purpose can be carried out in most body areas but are commonly employed in breasts, knees, neck, flanks, arms. Autologous fat transplantation has been utilized as an excellent filler material in cosmetic and reconstructive surgery for correction of soft-tissue defect and augmentation. Two different forms of AFT are commonly used: micro-graft and macro-graft. The fat of macro-graft is surgically removed from areas adjacent to the transplant site or in some cases from more distant areas. It is then trimmed to appropriated size for implanting to fill deformities of breast conserving operation(BCS) as well as symmetries after constructive breast surgery (Figure 1.3). The liposuction technique typically depicts the micro-graft of AFT (Coleman, 1997;

Coleman 2001). This form generally used as a cosmetic procedure often for rejuvenation (Figure 1.4).

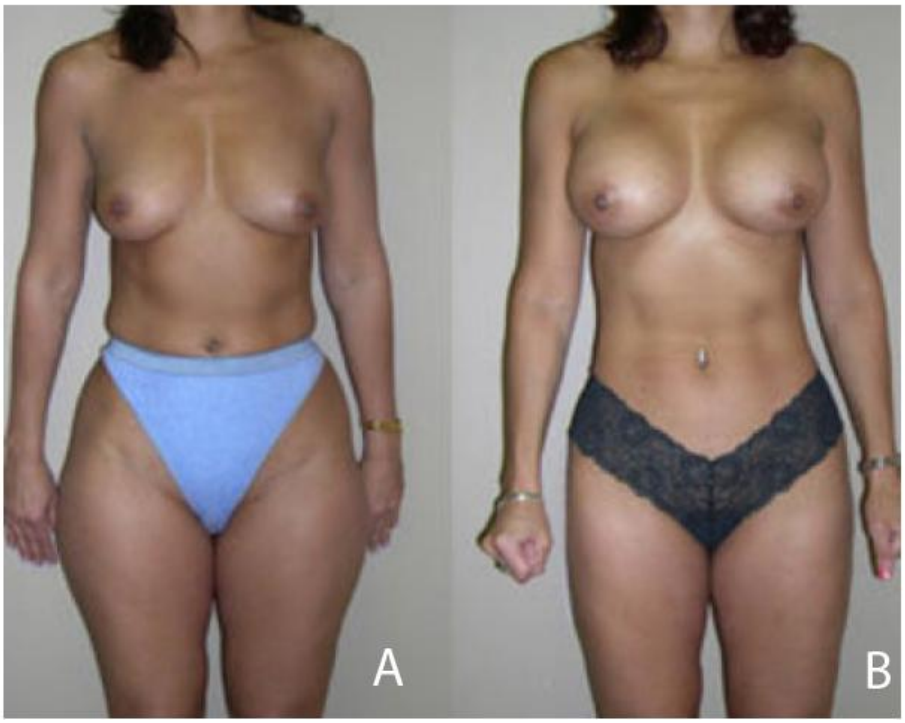


Figure 1.3 (A) Anterior view showing a 30 years old woman before fat injection.

(B) 3 months after injection of the breast area with 200 ml of fat tissue.

Figure 1.4 A 48 - year- old woman before and after 4 months injection of 2.4 ml fat tissue to the eye socket.

1.2.1 Patient selection

The optimal liposuction candidate possesses appropriate body weight, isolated focal and disproportionate adipose deposits (Coleman, and Hendry 2006). Liposuction should not be considered as a routine weight loss technique due to its invasive nature. Obese patients encouraged and motivated to adjust their nutrition and exercise regimens and view liposuction as a last resort. At the primary consultation, a complete history and physical exam should be carried out. For the surgical procedure, potential contraindications should be evaluated in terms of social, medical, psychiatric and surgical history (Flynn and Narins, 1999).

The pre-operative investigation must include assessment of the blood count, coagulation states as well as the basic metabolic laboratory tests. If the patient has any

signs of body dimorphic disorder or un-realistic expectations then surgery must be reconsidered. As with any surgical procedure, any non-prescription medicines must be cessated, for example, aspirin and aspirin containing medicines, alcohol, herbal remedies and vitamin. The postoperative plan should be discussed with the patient prior to the procedure. The blood volume normally represents 7% of total body weight of which red blood cells account for 40% and 60% plasma, blood loss must be quantified during liposuction surgery procedure, blood transfusion with red blood cells is required if more than 1500 cc of blood is lost.

1.2.2 Instrument and cannulas

The essential surgical instrument requirements to carry out liposuction are sterile cannulas and plastic tubing as well as and aspirating medical device (Bernstein, 1999). There are diverse cannulas available range in size, shape and diameter (Figure 1.5). Despite of the fact that smaller size cannula cause less trauma and afford the better control of instrument, the optimal cannula is selected depend on the treated area and preference of individual surgeon as well as cannula variability.

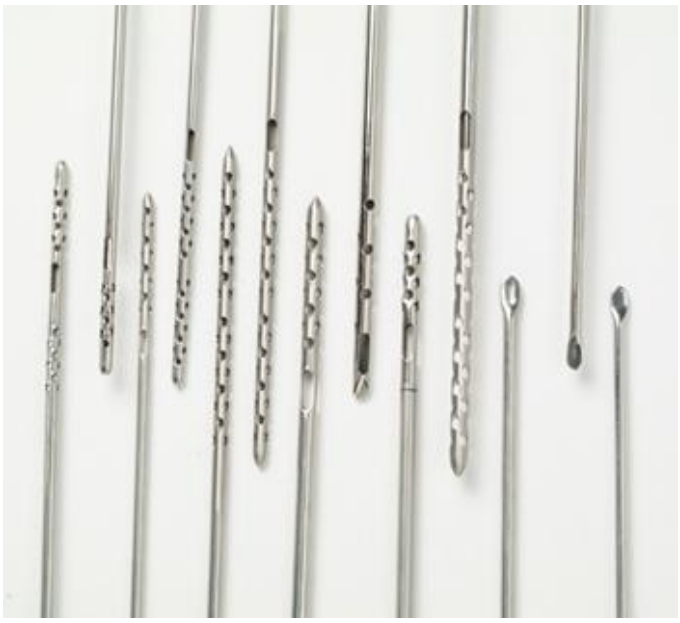


Figure 1.5 All kinds of liposuction cannulas are utilized for liposuction in cosmetic and reconstructive surgery.

1.2.3 Patient preparation on the day of surgery

On the day of the operation, a sedative should be utilized to prevent or reduce the anxiety of the patient. Preoperative medications should be prescribed during the two weeks before the operation.

1.2.4 Tumescent anesthesia

Tumescent anesthesia is a technique based on subcutaneous injection of a combination solution containing highly diluted lidocaine and epinephrine (Klein, 1993). This technique was pioneered by Klein, the method has significantly reduced

postoperative complication of liposuction, for instance, bleeding, bruising and the formation of hematomas and seromas (Lillis, 1988). The method of tumescent anesthesia includes the infusion of lidocaine with epinephrine diluted in physiological saline solution. The lidocaine is diluted to 0.05% to 0.1% and the epinephrine 1:1000,000 with 1000 ml of 0.9% NaCl solution with addition of 0.01 litter of sodium bicarbonate. This method allows a lidocaine dose up to 35mg/kg of body weight without toxicity.

At present time the technique has been developed in numerous studies with peak plasma concentrations of lidocaine taking place during the period between five and seventeen hours (Nordstrom, 2005). Coleman (1996) claimed a maximum dosage of lidocaine of up to 55 mg per kilogram of body weight without toxicity. A clear advantage of tumescent anesthesia is that elimates the known risks of general anesthesia. Tumescent anesthesia has been shown to be safe (Hanke and Coleman, 1999).

The technique is fairly simple and straightforward and carried out under appropriate sterile conditions. The tumescent anesthesia is infused though incision sites which made with A 15 blade. A blunt cannula is introduced through incisions to deliver the anesthesia from a prepared bag. The cannula is then moved from incision to incision in order to insert equal amounts of anesthetic solution. Depending on the patient's ability to handle pain and on where the procedure is taking place, the amount of tumescent anesthesia may differ.

1.2.5 After the procedure

After the surgical intervention has taken place, the incisions are covered with pads secured with tape, to prevent fluid leaking. Compression garments are also used after liposuction, in different forms and sizes, to reduce the risk for postoperative bruising and facilitate the elimination of anesthesia. These garments must be worn by the patient around the clock for the 2 weeks after the liposuction, during the night for the 2 weeks thereafter, and whenever the patient feels the need after 1 month. The first control, or follow-up visit, must occur 1 day after the surgical operation, with the next ones being fixed at the physician's discretion, after 1 or 2 weeks until 6 months, when usually the results will start to be seen.

1.3 Principle of autologous fat transplantation

1.3.1 Fat transplantation survival

Although the procedure of autologous fat transplant has not been standardized, methods and their consequences still need to be considered and analyzed, before use. In essence a full risk a assessment of all elements must be carried out before a method is selected.

Meschik (1944) found that when insulin rises metabolic activity and of adipocyte and decrease lipolysis (Skouge, 1992, Sidman, 1956, Smith, 1976, Solomom, 1980), vitamin E is an integrant factor in maintenance of adipocyte (Katoes et al., 1933). According to Hiragun (1980) insulin can transfer to adipocytes, and in the meantime, fibroblasts also can be induced by insulin to update lipid lost from lipolysis.

Adipocyte survival depends on techniques and instruments of harvesting and injection, while damage and cell death in a way rely on the size of instrument used to harvest and inject fat (Dolsky, 1987). A previous study found that the technical factors impact upon approximately 50% transplanted fat cell survival (McCurdy, 1995). On the other hand, other factors impact upon fat survival, such as, absorption, infection and embolism (Fisher, 2001; Yoon et. at, 2003; Fulton, 2003).

1.3.2 Indication for fat transplantation

According to (Skouge, 1992; Berdeguer, 1995) a simple and useful classification for autologous fat transplantation can be depicted into three categories: fill defects, cosmetic and non-cosmetic (Table 1.1).

Fill defects	Cosmetic	Non cosmetic
Congenital	Furrows(wrinkles)	Chordoma surgery
Traumatic	Ageing change	Lumbar laminectomy
Disease	Enhancement	Vocal cord scar
Iatrogenic		Myringoplasty

Table 1.1 Autologous fat transplantation can be used for different purposes.

1.3.3 Technique of autologous fat transplantation

There was a wide number of autologous fat transplantation techniques, however, the optimal method has not been yet been derived due to a number of diverse conflicting studies and operational experiences as well as the limitations of techniques.

1.4 Complications of autologous fat transfer

Despite of the fact that autologous fat transplantation is generally regard as a secure operational process, complications do occur after operational procedure. Most of the complications of autologous fat transfer inherent in the procedure are list below.

1.4.1 Absorption

The biggest problem that might appear in the case of fat insertion is the absorption of fat, which could render the entire procedure worthless if it reaches high percentages (Fournier, 2000; Khawaja&Shiffiman, 2010). The phenomenon takes places almost after every intervention, in percentages varying between 0 and 70% (Bircoll, 1992; Melin, 2001), and that is why authors recommend 30-50% over-corrections. The precision needed in the case of a cosmetic surgery operation is apparent from browsing the various reasons why the rate of fat absorption could get out of hand – replacement fat extracted from unsuitable areas (the upper abdomen or back), the use of over-pressurised machine for fat aspiration, or poor technique.

To avoid the danger of fat absorption, cosmetic surgeons recommend minimal facial expressions for patients that undergo facial fat transfer, and the avoidance of heavy physical exercise for the patients that received a larger quantity of adipose tissue. Table 1.2 showing the degree of persistence of fat.

Site	Longer	Shorter	Variable
Nasolabial folds		*	
Malar areas	*		
Cheeks			*
Marionette lines		*	
Glabellar frown lines			*
Transverse forehead lines			*
Lips		*	
Breasts	*		
Dorsum of hands	*		
Buttocks	*		
Calves			*
Tissues defects			*
Male external genitals	*		
Femal external genitals	*		

Table 1.2 Fat persistence degree according to site. Adapted from Khawaja & Shiffiman, (2010).

1.4.2 Infection

Another problem in cosmetic surgeries, common to all surgical interventions, is the danger of infections (Chajchir, 1996; Har-Shai, et al, 1996). This issue is related to the increasing appearance of mycobacterial infections (Figure 1.6).

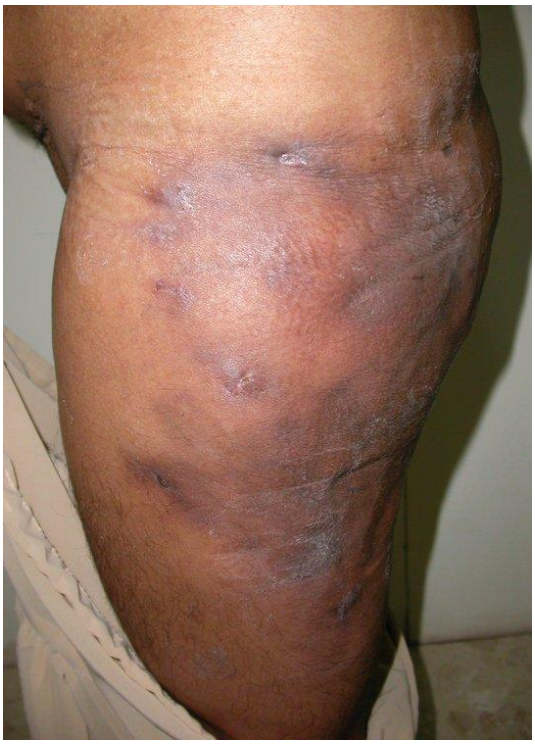


Figure 1.6 A typical infection after liposuction surgery showing multiple sinuses discharging serous fluid and peau de orange. Taken from Heack, P et. al (2009).

While during the operation a proper asepsis of the surgical instruments represents the single most important factor in deterring infections, the risk of infections can be lessened further if the patient follows a treatment of injectable and oral antibiotics both pre and post-operatorily. Strict sterile aseptie operating room technique,

perioperative broad spectrum injectable antibiotic as well as oral broad spectrum antibiotics 1 day before liposuction transplant and continuing postoperative for approximately 7- 10 days, can effectively prevent primary infection. Meanwhile complete blood count, fasting blood sugar and hepatitis B and C screening must be done preoperatively.

1.4.3 Embolism syndrome

According to (Gourlay T and Kamalthe, 2005; Platt, et al 2002, DiIllerud, 1991) mechanical liposuction can induce fat embolism after the procedure due to blood vessels damage through which some lipid globules can migrate into the venous circulation. A consequence of a post-operatoric infection is the appearance of septic pulmonary emboli (Walter JB and Israel MS, 1987; Feinendegen, et al, 1998; Alexander RW, 2001). In extreme cases embolism can lead to coma and death (Egido et al, 1993; Castello et al. 1999), and it is known that platelets in the thrombus eliminate thromboxane or 5-hydroxytryptamine, but it is not established whether the resulting spasms in the pulmonary vessels are the exact cause of complications (Walter, 1987; Fourme, Loubieres, Julie, Jaedin,1998; Castello, et al, 1999). However, some measures are known to reduce the risk of emerging embolism. For example, Shiffman (2001) recommends that fat transfers to the claves should not excceed 60-100 ml for each side.

In the worst cases, embolism can lead to mechanical obstruction, pulmonary infarction or progressive pulmonary hypertension (Alexander, 2001). Recent in vivo research (Gourlay and El-ali, 2005) showed that embolism in the lungs in 100% of animals after liposuction and that possibly there is some embolism in the brain.

1.4.4 Cysts, pseudocysts, and lipo necrotic cysts

The danger of cysts appearing increases with the amount of fat transferred, but it also depends on the body part where this takes place. Cysts are usually small, and normally occur on the face and breast (Castello, et al, 1999; Fischer, 2001) (Figure 1.7), however in the case of large fat transfers, especially in the breasts, moderate to large cysts may appear (Mandreakas, 1998). A 2 mm Bambi cannula can be used to aspirate cysts. In order to obliterate the cyst wall and prevent superficical cysts post treatment compression is used (Millard, 1994; Johnson G, 1992).

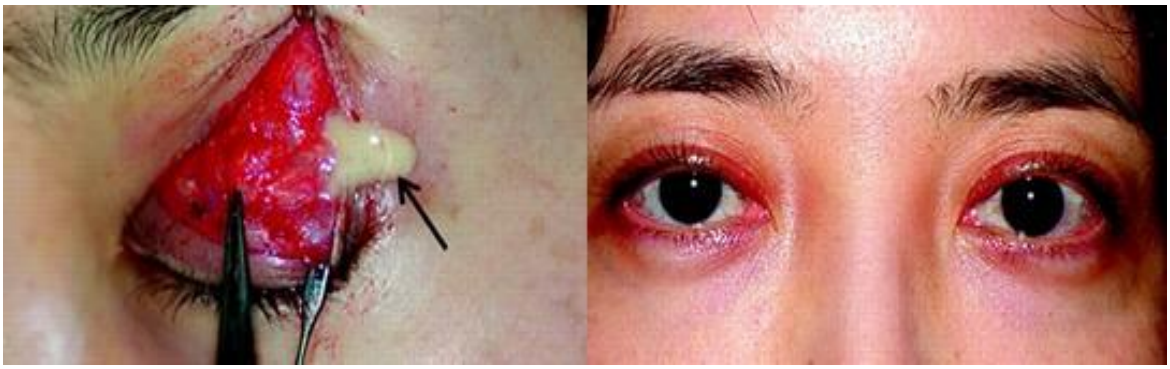


Figure 1.7 Indicates the cyts after the autograft injection in eyelid. Taken from Jean,D and Alastair, C (2006).

If calcification takes place in breasts cysts, they could be misdiagnosed as breast cancer (Bircoll, 1998; Shai-Har et al., 1996). Calcifications could also appear in fat deposits and in the most extreme cases can lead to ossification, especially if the patient presents hormonal imbalances or traumas in fat cells.

To distinguish the two forms of calcification, a full examination of their size and form, together with an analysis of their timing, position and character are needed to infer their causes.

1.4.5 Skin necrosis/sinus formation

If too much fat is injected, the phenomenon of over-augmentation and compression of the fat occurs, which may lead to skin necrosis and, in the grave cases, sinus formation. Intra-arterial fat injection can cause tissue necrosis (Hernandez-Perez, 1998). When an unnecessarily larger transfer of fat is carried out, or an excessive amount of fat is aspired, the resulting excessive compression applied to the area and the over-augmentation of fat may result into arterial or venous thrombosis, infarction of a number of anatomical structures and motor paresis.

1.4.6 Bloody fat, hematomas, and seromas

The lifestyle or health condition of patients represents an important factor in assessing the risk of post-operative complications. In the case of patients with liver or vascular disorders, or patients taking homeopathic medicine, there is a higher probability that hematomas or seromas (Khawaja, 2010). Patient condition, improper surgical instruments, an incomplete post-operative cooling or arterial and venous damages are other factors that influence the appearance of hematomas, in which case aspiration or compression can be carried out (Sheffman, 2002).

1.4.7 Iatrogenic injuries and pseudotumors

More generally, a number of nerves may be damaged during fat transfers, such as facial nerves branches (temporal, buccal, cervical, etc) or dorsal nerves in the penis (Salasche et al, 1988; Part, 1998), and pseudotumors may appear as a result of bruises and edema.

1.4.8 Lipomatous (hypertrophy) formation, symmetrical/asymmetrical lipomatosis

Complications can appear after longer periods of time (months or years) after fat transfer. This is the case of lipomatous formations in the lower lip, penis or dorsum of hands (Khawaja, 1999; Miller et al., 2008; Guaraldi et al., 2005). Symmetrical or asymmetrical lipomatosis may occur in the breasts, penis or the gluteal region. As in the case of embolism, the cause of lipomatosis and lipomatous formations and its mechanism is still not clear, but some authors have proposed fat hyperthrophy or hyperplasia as a candidate cause.

1.4.9 Skin pigmentation and fat migration

If sutures open postoperatively and fat leaks out, darker complexioned patients may experience high levels of skin pigmentation, which can be treated with sun creams and hydroquinone (Danesh HV, 2001). Due to its self-limiting character, skin pigmentation does not generally lead to graver problems.

Fat migration occurs if mechanical actions are exercised on an already over-injected body part (Clleman, 1994; Fournier, 2000; Khawaja & Shiffiman, 2010). Sleeping on

the specific body part or muscles, in particular, can be damaging for the forehead, if fat is injected there.

Despite the number of problems that may result after a cosmetic surgical intervention, autologous fat injections have become an increasingly popular procedure. Because the chances of running into post-operatory complications do not seem significant, patients have increasingly refered to cosmetic surgeries and their satisfaction has increased. Moreover, it is hoped that developments in medical technology would reduce the risks still further.

Chapter 2 - Technology for fat concentration

2.1 Centrifugation of fat

Since 1980, centrifugation has been utilized in concentrated aspirated fat, to remove blood, fluid and oil in liposuction as part of cosmetic surgery (Shiffman, 2001; Puckett, 2004, Smith, 2006; Kurita et al., 2008). There is a varying clinical experience with this technique. Some physicians assert that centrifuging fat tissue to remove blood and other lipids can enhance the quality of fat for injection, whilst, others claim it is not necessary to centrifuge fat because there is no obvious advantage (Ellenbogen, 1986; Toledo, 1991; Zocchi, 1991). Toledo (1991) stated that spinning the autologous fat at about 2000 rpm for approximately 1 minute to eliminate the undesired solution, enhances the fat tissue for transfer. In his article Uebel (1992) reported that centrifuged fat tissue, five minutes at 10,000 rpm to obtain the pure fat material, comprised approximately 5% intact adipose, collagen fibres and cell residues (Figure 2.1). Brandow and Newman (1996) observed that centrifuging the fat tissue did not modify the natural histological structure of adipocyte. According to Fulton et al (1998) centrifugation of fat harvest at 3400 rpm for 3 minutes, improves fat tissue grafts for small volume transplants, but not for large volume.

some studies have shown that excessive centrifugation may damage and degrade intact adipocytes (Shiffman, 2000; Boschert et al.), however appropriate centrifugation can enhance total amount of transplanted fat and enhanced graft take (Coleman, 2001; Kurita, 2008).

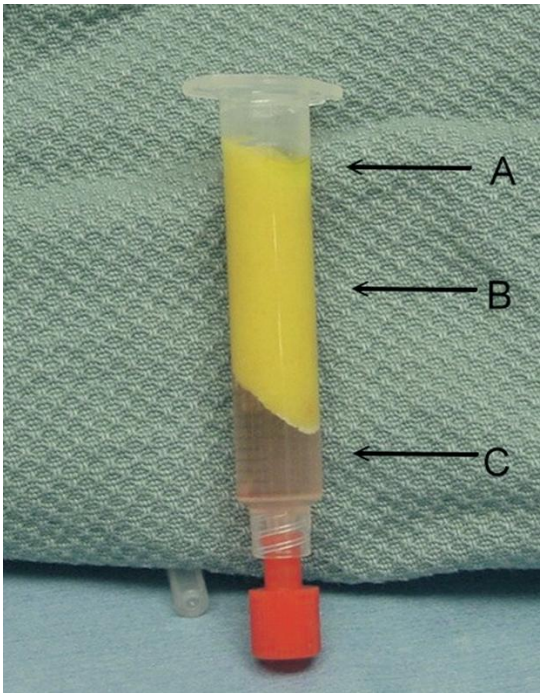


Figure 2.1 Processed 10 ml liposuction of harvested fat showing 3 layers—: oil (A), prepared transferred fat (B), blood-aesthetic mixture (C). The photo was adapted from Kurita (2008)

2.2 Current techniques of fat transplantation

Autologous fat transplantation is an important technique for cosmetic and reconstructive surgery. However, in some cases, the operation could be repeated one or more times to obtain satisfactory results. Although it is safe in the hands of qualified and experienced surgeon, nevertheless, a number of complications may occur after AFT. Over the past half century a large number of technologies have

evolved to meet the delivery challenge associated with autologous fat transfer. There are a number of approaches to this, and no, one standard clinical technique exists. The current techniques available to practitioners today include:

2.2.1 The LipiVage System

The LipiVage fat harvest, wash, and transfer system is a disposable, medical device developed by Genesis Biosystems Inc that is shown figure 2.2 and figure 2.3 (Robert E et al, 2007).

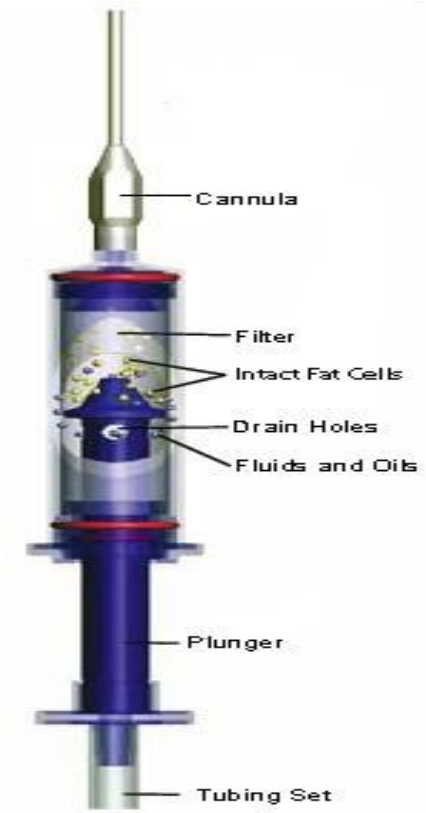


Figure 2.2 The LipiVage fat harvest system in 3D which shows the form of the device. Taken from www.genesisbiosystems/products/lipivage-processing/363.short.

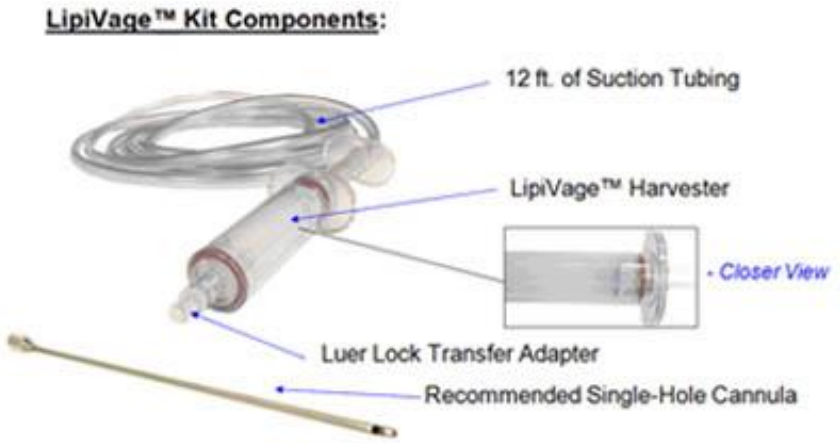


Figure 2.3 The components of the LipiVage fat harvest system. Taken from www.lipivagemedical/function/components/13.

This device harvests fat grafts at low vacuum level, washes fat grafts within a closed system, and avoids centrifugation, decant, or other unnecessary handling steps. It represents an improvement upon traditional fat transfer (Toledo, 1996; Guyuron & Majzoub, 2007). While inside, the filter and low vacuum are able to gently clean and concentrate the adipose tissue. Other unwanted components are aspirated by vacuum into an attached waste canister within the device. The end result yields intact and concentrated fat grafts which are immediately ready to be utilized for injection (Figure 2.4). This system has been shown to be gentle on fat cells during harvesting and processing, quick, simple to use (Robert E et al, 2007). The clinical procedure is simple: a mixed solution of fat suspension is infiltrated at the donor site by a blunt Lamis infiltrator through a small incision. An appropriate cannula, connected to the

LipiVage system, is inserted in the donor site, and then fat tissue is harvested through a sterile filtration chamber at low vacuum pressure (Figure 2.5). This device is designed for single use only. It attaches to the liposuction pump and vacuums adipose cells. In addition, it uses an enclosed system for cleaning fat graphs (without the use of a centrifuge) and minimizes direct contact with expunged content. The driving mechanism for this device is a low level vacuum with the concentrate constituents being controlled by gross mechanical filtration.

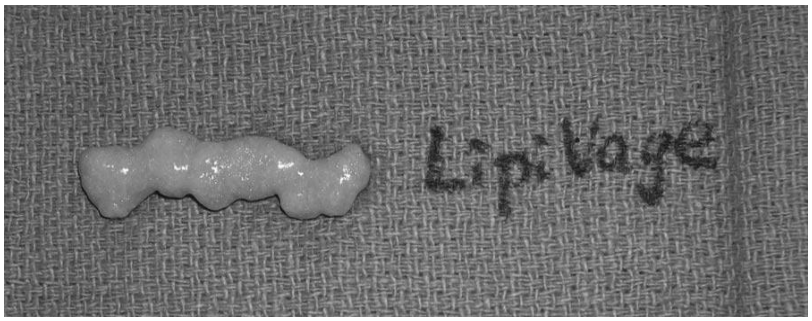


Figure 2.4 Gross appearance of fat grafts harvested with the LipiVage system. Adapted from Ferguson, et al (2008).

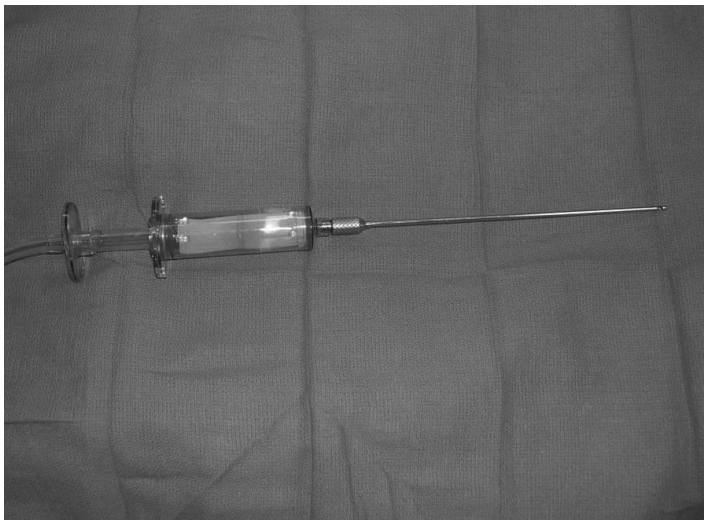


Figure 2.5 The LipiVage System is a closed system that combines harvest, wash, and transfer in one setting. This system however needs to be connected to a vacuum source in order to function. Adapted from Ferguson, et al (2008).

2.2.2 The Celution System

The Celution system is designed by Cytori Therapeuties Inc. (Figure 2.6) has the ability to process around 250 mililiters of liposuction tissue within approximately one hour. The system can be used in an aseptic manner (within the single use Celution set or simply saline and lactated Ringer's)

Figure 2.6 The Celution System which can automated islolation of autologous fat. Adapt from Duckers, H et al (2006).

Divided into two separate components (dedicated to washing/digestion and concentration of cells, respectively), it employes a manual isolation system similar to that of (Duckers, et al, 2006) and Zuk et. al., (2001). Potentially carried-out either at the bedside or within a primary coronary intervention, its use involves first the harvesting of a small adipose tissue area through a small stab incision and the

subsequent washing of the tissue to remove debries (Morizono et al., 2003; Kern et al., 2005). Prior to the procedure's initiation, a Toomey syringe and cannula is placed , this only required local anestesia.

Following a process involving the tissue's partial digestion, any released mononuclear cells would be moved to the centrifugation vessel, where a reccurrent cycle of washing and centrifugation would allow the localization of the targeted population of cells within the mashine's output chamber (Garcia-Olmo, et. al., 2003; Yoshimura, et al, 2005). Through injection, the population of cells are applied to the patient imediately (Figure 2.7).

Figure 2.7 Adipose tissue is harvest, processed and injected using the Celution system which is fast and enhanced the effency.

2.2.3 The Coleman System

Coleman first stated his technique which utilizes syring, cannula and centrifuge for structural adipose graft (Coleman, 1997) (Figure 2.8). This system involves the infiltration of a solution made from mixing 0.5% lidocaine with epinephrine (1:200000) and lactated Ringer's solution through an incision (using a blunt Lamis infiltrator) in a proportion of 1fat harvested graft/1 cubic centimeter of sollution (Coleman, SR, 1997, 2002). The incision could also be used for extracting the grafts using a 3mm blunt-tip canula. This is attached to a syringe (10cc Luer- Lok) (Figure 2.8).

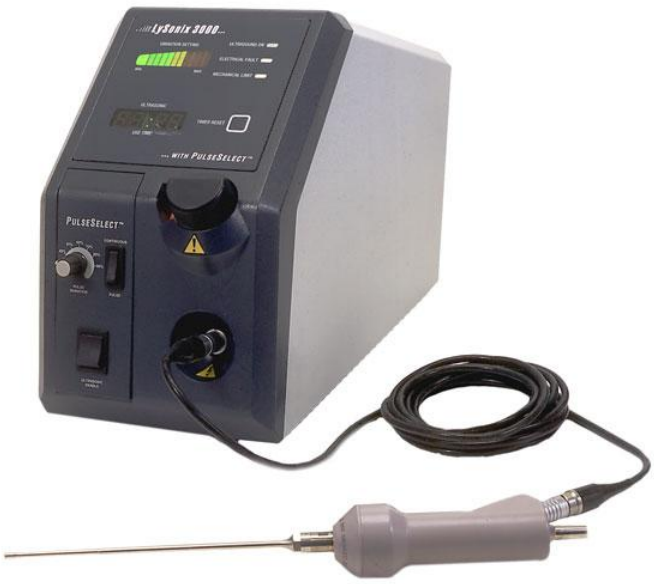


Figure 2.8 The power assistant Coleman system is a highly refined concept in instrumentation for removing and preparation of aspirant. Adapt from Jeremy R, et al (2011).

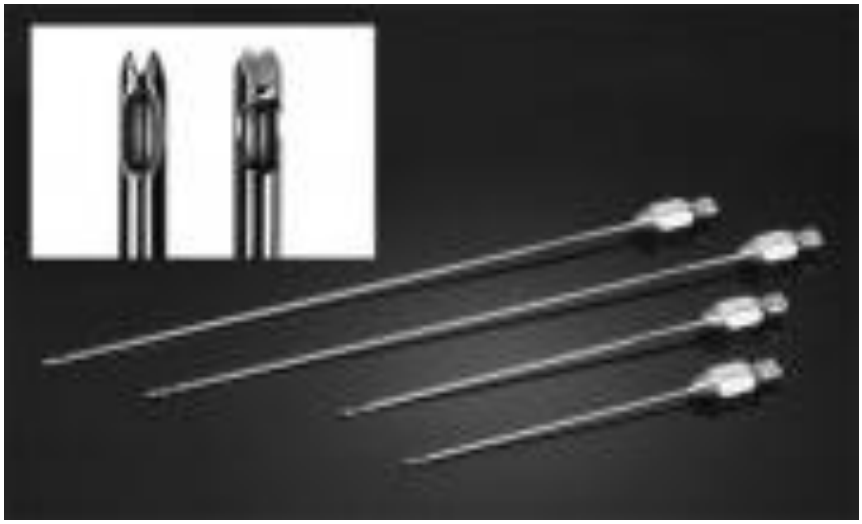


Figure 2.9 The specially designed cannula connect with the Coleman system is put on incision for used to harvest fatty tissues. Adapt fromKarol, A and Gutowski.2009.

Through the additional use of another syringe for negative pressure, the first syringe would become full of tissue and the cannula would be discarded. Through the use of a plug (Luer-Lok), the syringe could be retracted and placed into a centrifuge (3 minutes at 3000 rpm) (Coleman, 2001; Roberts, et al, 2006). Following this process, the fat concentrate is decanted and drained for injection (Figure 2.10) .

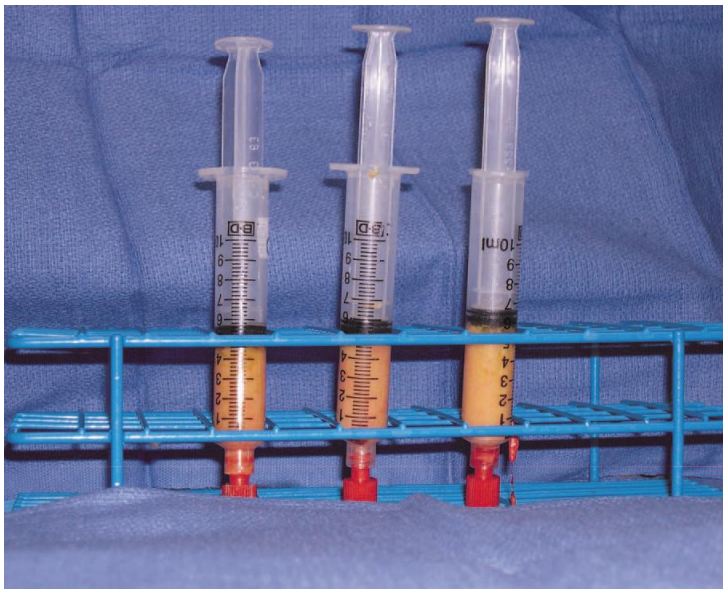


Figure 2.10 Fat grafts were harvested and processed with the Coleman technique, and spun at 3000 rpm for 3 minutes. After centrifugation, both upper and lower levels of components were removed, and the remaining autografts within syringes were subsequently injected. Adapted from Pu, et al (2008).

2.2.4 The Viafill System

Representing a state-of-the-art system for processing autologous fat, the Viafill system (Figure 2.11, Figure 2.12) uses for harvesting a manually-operated 20cc syringe and a lubrified cannula for aspiration. The 20 cc harvest syringe of the Viafill system is shown in Figure 2.13. The system was designed to minimize damage to adipocytes viability during harvest and reinjection, and to maximise the number of live adipocytes transplanted. The Viafill system can address most of current AFT channenges by optional manipulation, trauma and exposure to air during the AFT procedure.

Figure 2.11 The Viafill System shows liposeCorp, and patented system for the aspiration, harvesting, filtering and reinjection of autologous fat. Adapted from www.viafill.com/about-viafill.

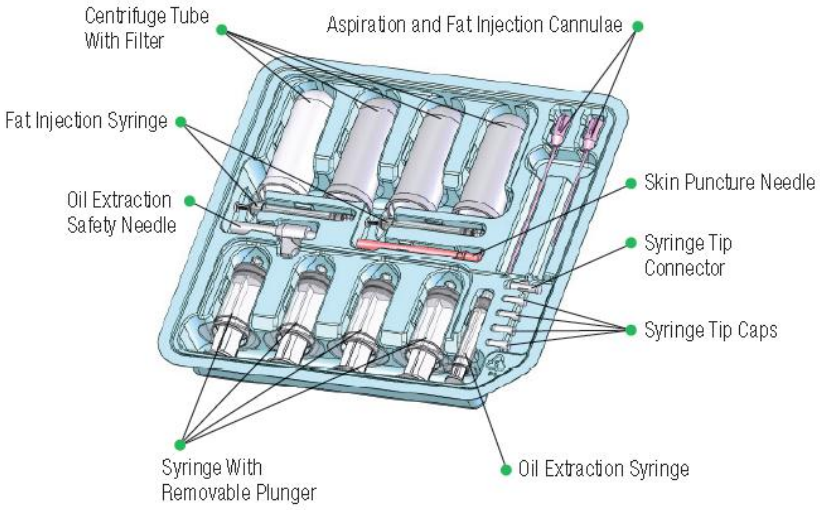


Figure 2.12 The components of the patented system of Viafill system. Adapted from www.viafill.com/viafillcomponents/201/htm.

Figure 2.13 The Viafill short, broad 20-cc harvest syringe is operated manually, avoiding the generation of high negative pressure and cell damage that typically results from mechanical aspiration. Taken from http://vimeo.com/7857648.

The Viafill is similar to the Coleman system in terms of using the syringe as a chamber to be placed in the centrifuge, but with a variety of additional components e.g. a removable plunger, a rubber stopper, and a continuously open syringe tip – for allowing the expulsion of blood and debries to reduce the risk of air contact. (Neuyen A et al., 1990 ; Boschert et al., 2002). This centrifuge (Figure 2.14) uses a swinging arm (moves 90 degrees) and applies to the cell mass a 50 grams force for 2 minutes attempting to minimise potential cell damage and increase the amount of usable recoverable cells.

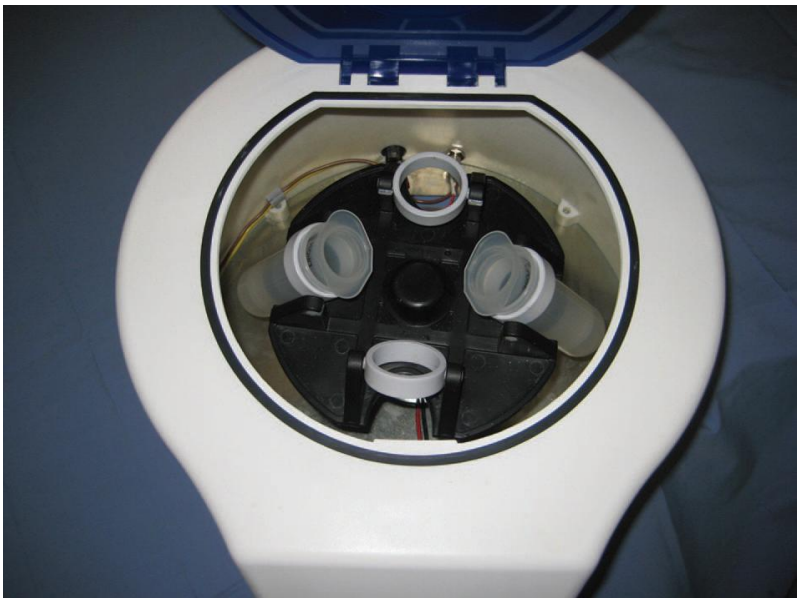


Figure 2.14 The centrifuge prototype of the Viafill system prototype show two filters and syringes. Taken from Rigotti, M,et al. (2009).

The Viafill plunger arm (Figure 2.15), coupled with a needle and syringe, can be used to perforate the Viafill rubber stopper and extract oil without damaging the viable cells from the bottom layer (Figure 2.16). After the reinjection, the physician can actually opt for using only the cells from this layer, which usually contains the highest percentage of viable units. The procedure for reinjection can be carried out through the harvest syringe or through luer-to-luer transfer device, both with minimal exposure to air.

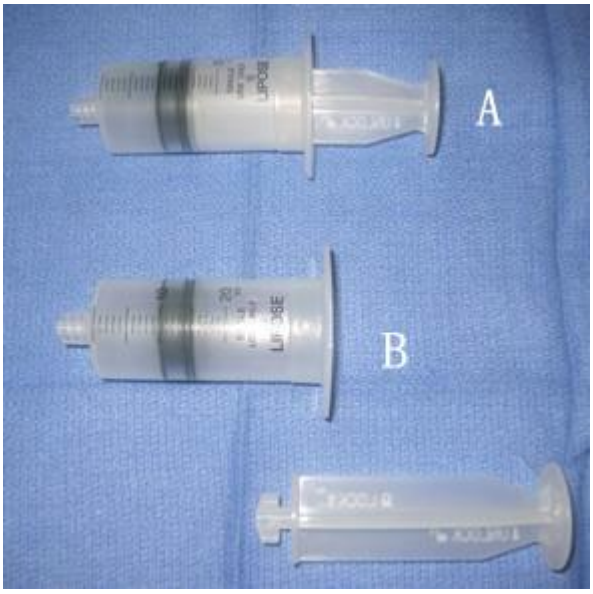


Figure 2.15 (A) A syringe from the Viafill system with a plunger handle in place. (B) A syringe with the plunger unlocked and removed. Adapted from Lauren. C, et. al. (2010).

Figure 2.16 Viafill syringe and centrifuge tube after centrifugation. Note that the heavier aqueous layer and erythrocytes have passed out of the syringe through the filter and the adipose and oil layers remain. Taken from Lauren. C, et. al. (2010).

2.2.5 Tulip System

The Tulip Medical syringe used to aspire adipose tissue is equipped with particularly smooth cannulas. Its luer-lok connection, coupled with the same type of syringes, play an important role in maintaining equal vacuum forces in the microcannula system during the lipoaspiration procedure. Moreover, the luer-lok connection acts as a stabilizing base when very small, flexible cannulas are used within the tissue (Figure 2.17).

Figure 2.17 Tulip medical closed syringe micro-cannula system for liposuction and fat injection. From left to right: anaerobic transfer (Super LuerLok); cell friendly microcannulas; mechanical injector gun; syringes with Locks In place respectively. Adapted from Alexander, R (2010).

The harvesting cannulas of Tulip system are designed to actually remove the fat tissues from the deep and intermediate layers. It is recommended that appropriate diameters of cannulas are utilised smaller for thinner and lower percent body fat

patients and larger for the majority of patients. Tulip Syringe Locks are designed for use on luer-lok syringes to hold the syringe plunger in a fully drawn position during the vacuum application. While in the locked position and all air removed from the device, the surgeon can apply constant vacuum pressures when moving the cannula through the adipose layer.

Tulip anaerobic transfers are utilized to facilitate loading of treatment syringes for both combining platelet-rich plasma (PRP) and fat grafts into one syringe (Figure 2.18 a, b). The tulip injector gun is designed for placement of treatment mix. When AFT is added to the PRP, the injection material density is increased. Single trigger pull provides precise volumes of solution to be placed with less pressure required (Figure 2.19).

Figure 2.18 a Shows anaerobic transfers, Super Luer-Lok attachment. The left is Cell-Friendly (non-disposable) the right one is disposable. Adapted from Alexander, R (2010).

Figure 2.18 b Anaerobic transfers (Luer-to-Luer) for loading syringes. Adapted from Alexander, R (2010)

Figure 2.19 Mechanical injector gun is prepared for injection. Adapt from Zul, P et al (2001)

In opting for using a cell-friendly microcannule within a Tulip system, the damage to the adipose tissue is reduced due to the their polished, smooth interior, whereas the external cannula anodizing process enables a smooth passage within the adipose base under the skin (Figure 2.20). This type of auto cleavable microcannule represents the most common design used in autologous fat grafting interventions. However, because of the difficulties in sterilizing procedures due to the small size of the microcannulas, the cell-friendly microcannule can be replaced by a more attractive disposable alternative. The gel coated disposable option comes in an already sterilized wrap and features the same size-related advantages of the non-disposable microcannulas. Moreover, they are coated in a hydrophilic gel for maximum ease of passage through adipose tissue.

Figure 2.20 Tulip medical disposable microcannula system shows all kinds of size cannula for liposuction and AFT injection. Adapted from Alexander, R (2010).

2.2.6 Cytori's PureGraft System

Cytori's PureGraf system (Figure 2.21) presents a fat processing technology which is developed to prepare autologous fat grafts in a closed and sterile system.This is achieved in the PureGraft system which has quickly became the benchmark of the current cosmetic and reconstructive fat grafting trend due to its efficient and quick production of graft tissues for autologous fat grafting interventions (Anitua, 2001; Baker, 2010). Moreover, if its use is combined with that of Celution (R) 800/CRS, the production times and processes are further reduced.

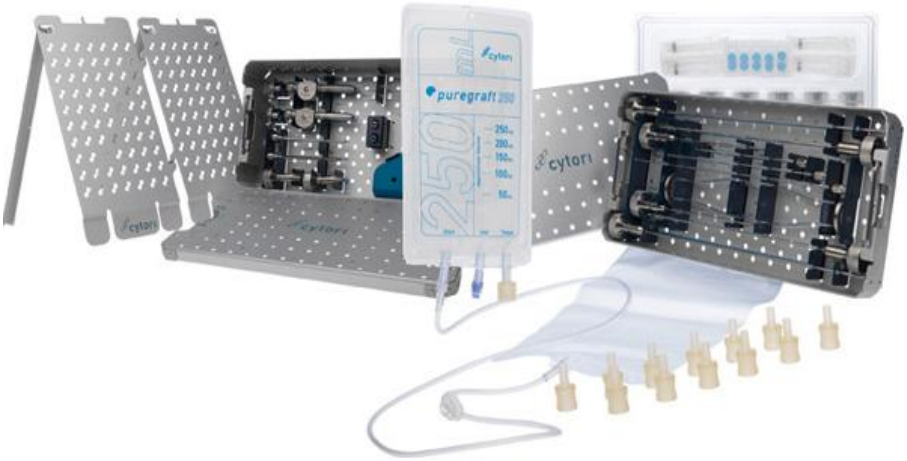


Figure 2.21 Pure Graftsystems that optimize the entire fat grafting process, from adipose tissue collection to graft delivery.

The key element that sets PureGraft System apart from competitors is its membrane-based filtration performed with speed, ease and precision, that smoothly removes excess fluids, blood cells and other debris. It takes only 15 minutes to purify a fat

graft of up to 250 ml through the PureGraft System's membranes (Garcia, et al, 2005; Barret, et al, 2009). The first membrane that remove loose blood cells, free lipids, fluid and other debris resulting from the liposuction process. The second membrane directs the flow of the debris and allows for the draining of the contaminants into a waste bag.The tissue is washed twice with 150 ml of processing solution. This washing step allows physicians to control the hydration level of the graft. The right balance of hydration can be important in addressing the varying quality of graft recipient sites and ensure graft quality. After the washing and draining are completed, the concentrated graft tissue can be extracted for use in autologous fat transfers (Figure 2.22). The device relies on a vacuum system to facilitate fat concentration.

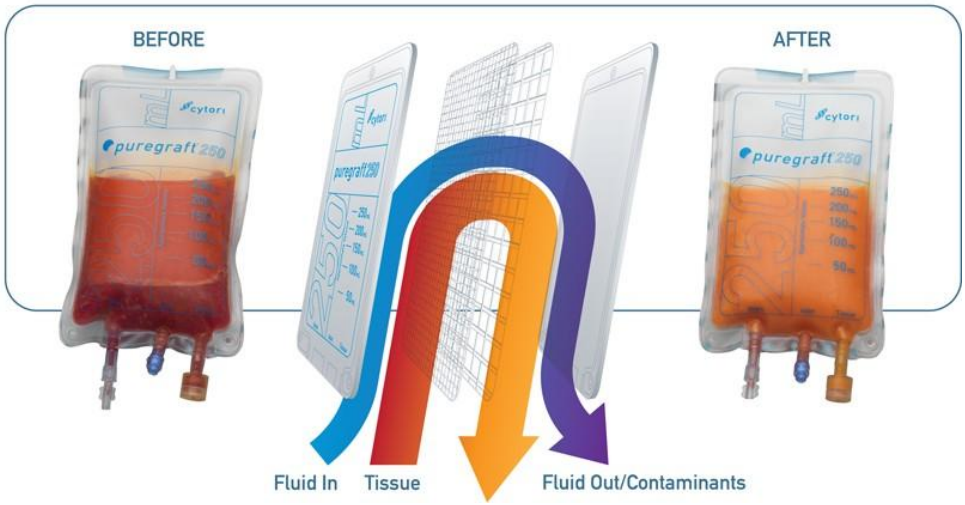


Figure 2.22 The Cytori's PureGraf system uses bilaminar flow filtration technology to process fat tissues.

Adapted:http://medgadget.com/2010/08/cytoris_puregraft_250pure_system_gets_ce_approval.html

2.3 Ratchet gun for injection

Neuman and Levin (1987) invented and firstly reported lipo-injector, a device with gear driven plunger to inject fat tissue. Agris (1987) reported his achievements in scientific research: ratchet-type gun which can precisely control autologous fat deposition. The trigger of the gun allows to pull 0.1ml deposition each time. Asadi and Haramis (1993) stated that he used the gun to inject fat with a 10 ml syringe. A lipotransplant gun was utilized to inject fat (Berdeguer, 1995).

Chapter 3-Thesis Objectives

The present work is focused on the development of an alternative technology for use in autologous fat transfer procedures. A novel and versatile fat concentration device which addresses the major clinical issues which include:

1. Minimal tissue handling
2. Closed, sterile processing environment
3. Minimal need for complex machinery
4. Preferable a “one-size-fits-all” technology.
5. Quick and efficient tissue handling
6. Ability to process a wide range of tissue volumes

The Bioengineering Unit at the University of Strathclyde has recently developed a novel technology (HemoSep) for the separation of plasma from whole blood for cardiac and trauma procedures. The overriding objective of the present work is to determine whether this technology, delivered in a different format can be applied to autologous fat transplant, resulting in a fast, efficient and inexpensive approach to this clinical challenge.

Chapter 4- Device design

The University of Strathclyde has recently developed a novel blood separation device, haemoconcentrator (HemoSep) with in collaboration with Brightwake Ltd. This device is based upon a membrane controlled superadsorber technology that offers a simple, inexpensive approach to haemoconcentration after CPB, with the added advantage that the device produces a gelatinous rather than liquid effluent which is both safer in terms of contamination risk, and easier to dispose of than conventional liquid effluent. In discussion with Brightwake Ltd, it has been suggested that a similar superadsorber, membrane controlled approach may be suitable for concentration the production of liposuction into a fat concentrate for cosmetic and reconstructive surgery.

4.1 The Principles Underpinning the HemoSep Technology

The HemoSep device concentrates blood cells by removing the fluid component of whole blood, the plasma, from a pooled volume of blood. The technique for removing the plasma from the blood product, leading to concentration of the cellular components is fairly simple, but involves a number of critical steps and controls (Figure 4.1).

Figure 4.1 The HeoSep technology consists of 3 main components,1.theHemoSep bag2. The Fixed Rate Orbital Shaker and 3.The Transfer bag.

4.1.1 The HemoSep Bag

The HemoSep bag element of the system is the active processing section of the device. It consists of a PVC blood bag with a polycarbonate membrane bag suspended within it, within which is a sheet of superadsorber material. The polycarbonate membrane material employed in the device has a 2 micron pore structure (Figure 4.2).

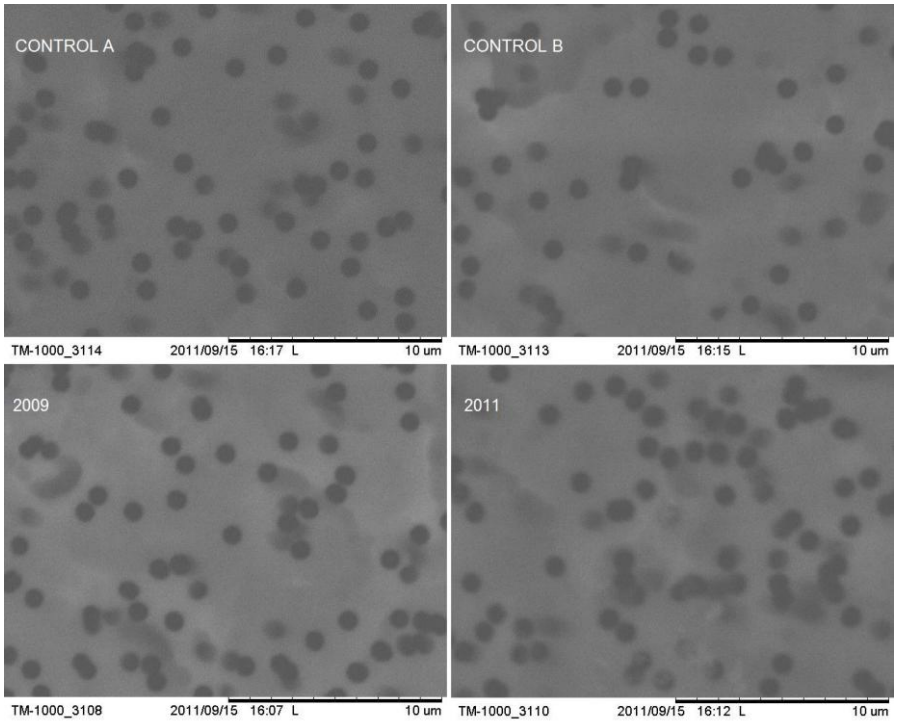
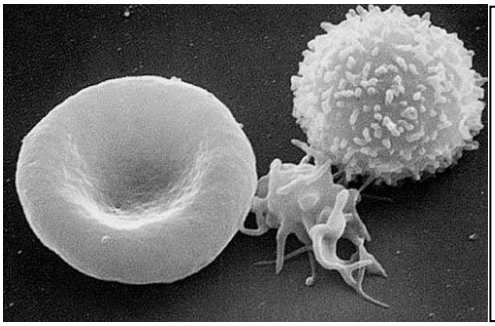


Figure 4.2 Scanning electron Micrograph of the surface of the polycarbonate control membrane. The 1 micron pore structure can be clearly seen form this image

The polycarbonate membrane is the control membrane of the system and the 2 micron pore size for this important element of the device has been selected to ensure that no cellular components of the blood product can pass into the superadsorber during use. The 2 micron pore size is small enough to permit efficient fluid transport into the superadsorber, but is too restrictively small to permit the passage of cells, even the smallest cells the platelets (Figure 4.3).



Red Blood cells – 7-10 microns

WBC's – 10-12 microns

Platelets – 2-3microns

Figure 4.3 Typical size range for blood cells. Note the platelets, the smallest of the blood cells, are typically 2-3 microns in size.

The 2 micron pore size of the polycarbonate control membrane is therefore sufficiently small to prevent the passage of platelets and all other cell species into the superadsorber section of the device. Some platelets may be smaller than 2 microns and it is possible that some of these platelets will pass through into the superadsorbersection, however, these should be in small numbers. The other blood cell species, the red and white cells will be spared by this system by mechanical filtration.

The superadsorber element of the HemoSep bag system is the driving force behind the transport of fluid (plasma) from the blood pool, through the control membrane and into the superadsorber section.

Once the blood product has been processed, the plasma which has passed into the superadsorber phase during the process is locked into the superadsorber forming a gelatinous matrix (Figure 4.4).

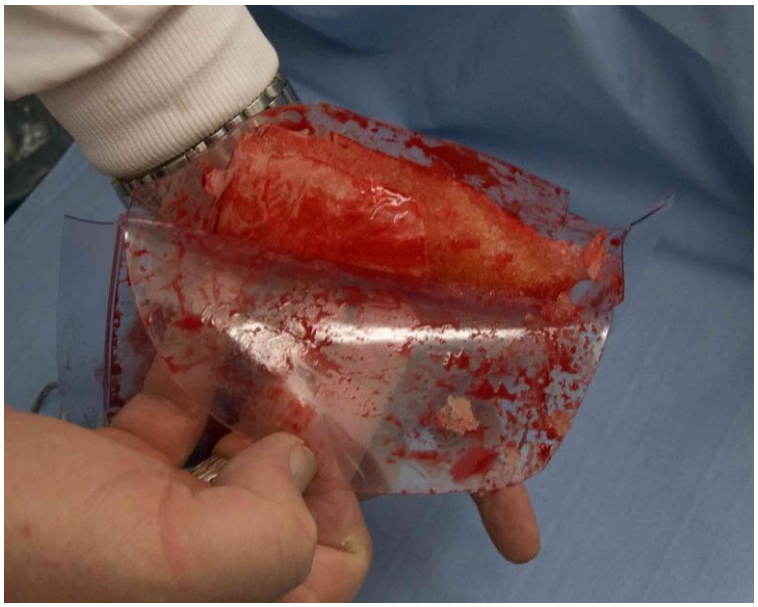


Figure 4.4 The plasma which passes across the control membrane into the superadsorber forms a gelatinous matrix which cannot pass back into the blood reservoir. The matrix can be seen here as a yellow gelatinous material in the centre of the device.

4.1.2 The Fixed Rate Orbital Shaker

Although the HemoSep device is capable of concentrating blood cells passively, the time taken to achieve the desired concentration is considerably shortened by employing some agitation of the device. The reason for this is that as plasma is drawn across the wetted control membrane by the superadsorber, cells can be deposited in the pores reducing the number of pores available for fluid transport. Laboratory

studies have demonstrated that agitation of the device during processing eliminates the deposition of cells in the pores and ensures that pore patency is maintained. Using the orbital shaker reduces the processing time by around 33%. A fixed rate of 120 cycles per minute has been determined as the most efficient frequency for the system, leading to maximal improvement in the exchange performance with no impact upon cellular damage.

4.2 Nonclinical Performance Studies of HemoSep device

Early performance tests of the HemoSep device were performed using fresh heparinised bovine blood with hematochrit adjusted with saline solution to match common clinical values. In addition to testing the performance of the device with bovine blood, tests were carried out using freshly donated human blood to confirm the function of the device under near-clinical conditions.

4.2.1 Bovine Blood Tests

Fresh heparinised bovine blood was diluted using saline to a packed cell volume (PCV) of 20%. A 500ml volume of blood was then injected into the HemoSep device after the device had been wetted and activated with 200ml of saline. The blood was then left to incubate in the HemoSep device for a period of 40 minutes. Blood samples were taken from the device at 5 minute intervals and the PCV measured using centrifugation (Figure 4.5).

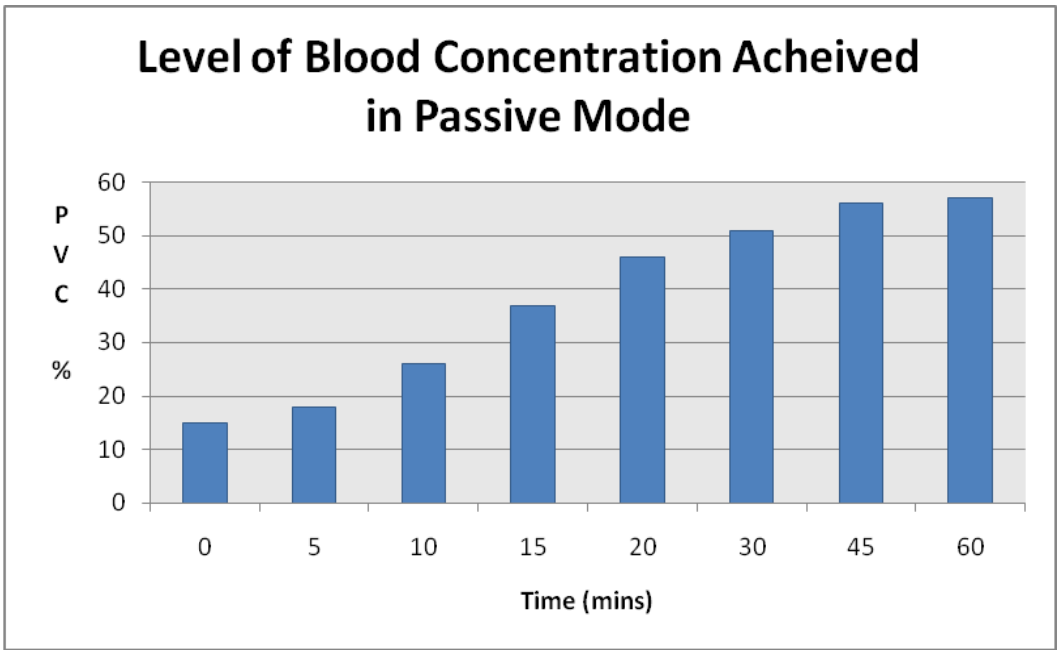


Figure 4.5 Effect of passive incubation on packed cell volume (PVC) in fresh heparinised bovine blood haemodiluted to 15%. The clinical target level of PVC (35-40%) was achieved in around 15 minutes.

In an effort of reduce the processing speed of the device, the addition of an agitation step was introduced. The agitation was provided by placing the blood filled HemoSep device onto an orbital shaker platform, operating at 120 cycles per minutes. This additional process reduced the blood processing time (time to reaching desired clinical values) by up to 30% (Figure 4.6).

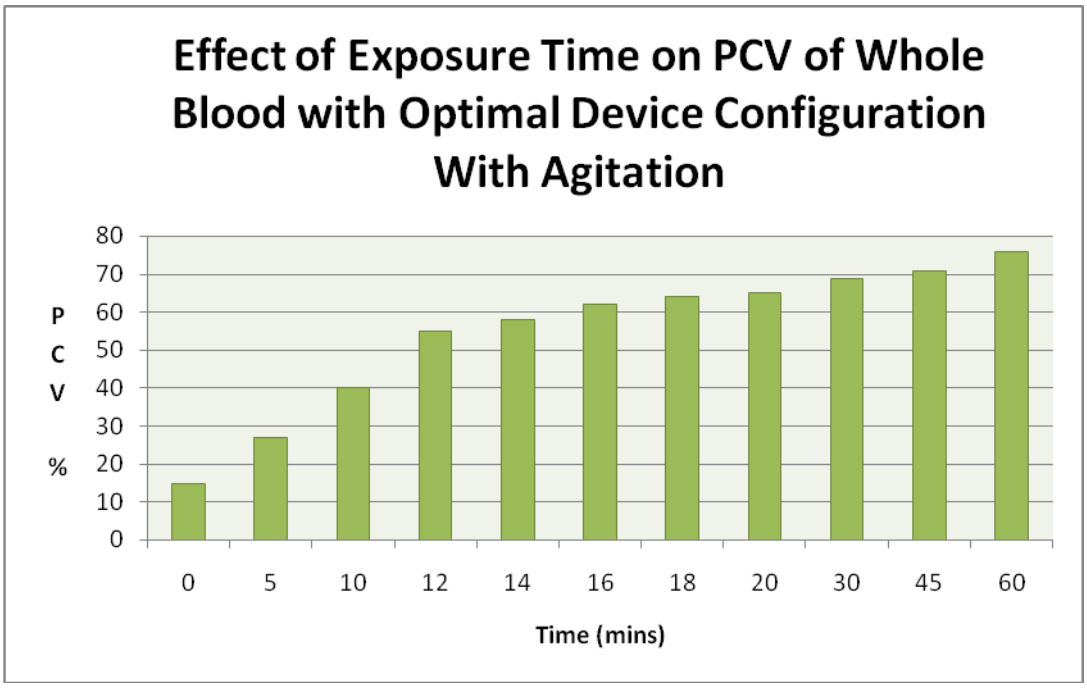


Figure 4.6 Effect of active incubation on packed cell volume (PCV) in fresh heparinised bovine blood haemodiluted to 15%. The clinical target level of PVC (35-40%) was achieved in around 10 minutes. This represents a significant reduction in processing time which may be important in the clinical setting.

Through these bovine blood studies an number of factors were clarified.

1. The device was shown to function very well in terms of raising the PCV of haemodiluted blood products to acceptable clinical levels.
2. The addition of agitation during the concentration step reduced the processing time, most likely by effectively scouring the control membrane surface with blood during processing, resulting in the maintenance of pore patency.

However, these early studies did highlight the importance of pore size on the quality of the blood product generated by the device. These early studies were performed using a 5 micron pore size membrane which was very effective in ensuring low processing times, but resulted in the removal of blood platelets from the blood product. This is due to the fact that blood platelets are smaller than the effective size of the pores and pass from the blood phase into the superadsorber phase. Further work using a 2 micron control membrane resulted in as a reduction in the level of platelet removal, but, given that the number of pores remained the same, resulted in a reduction in the effective open area of the device which impacted negatively on the processing time (Figure 4.7 and 4.8).

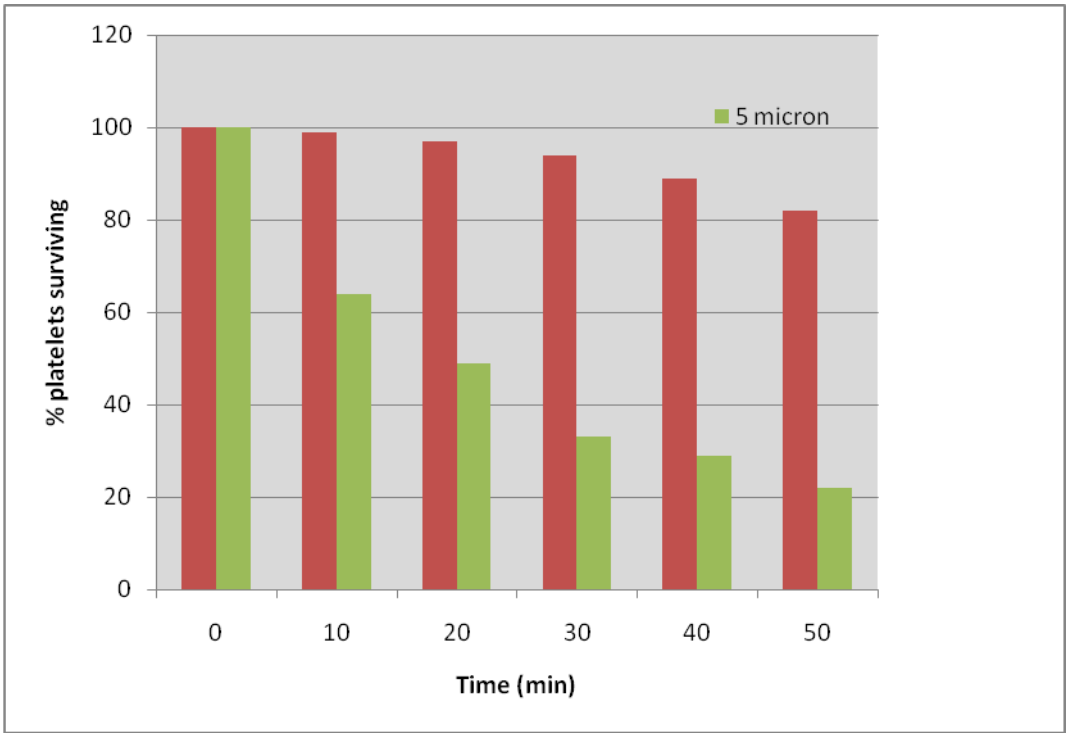


Figure 4.7 Effect of pore size on platelet survival. The 2 micron pore size control media (red) was associated with less that 20% platelet loss over 50 minutes. This compared to 76% platelet loss over the same time period with the 5 micron media (green).

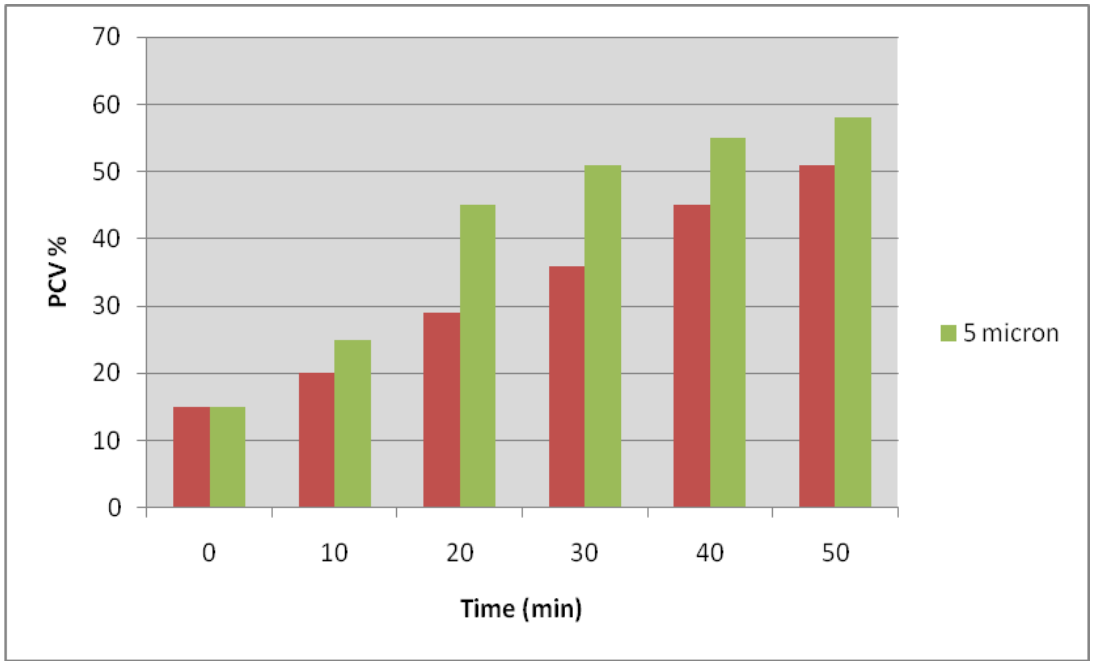


Figure 4.8 Effect of control membrane pore size on processing time. The smaller pore sized has the effect of prolonging the processing time to reach clinically desirable PCV levels. The time take to reach these levels is increased by in the region of 30% from 20 minutes to 30 minutes.

These data confirm that the 2 micron control membrane, although increasing processing time, spares platelets, a very important element in the maintenance of hemostasis in post-cardiotomy patients. The slightly longer processing time remains within clinically acceptable levels. The 1 micron control membrane is therefore the configuration selected for the clinical device and was the basis of all further studies.

4.3 Studies with human blood

Similar studies to those carried out in bovine blood were performed on fresh heparinised human blood by the National Blood Service, Brentwood,Essex. The focus of these studies was to determine whether the results obtained from the study of the HemoSep device with bovine blood was reflected in studies with human blood. Although there is little by way of cell size difference between bovine and human blood, there is always a possibility of inter-species differences in terms of testing a novel medical device. These studies utilised a fixed processing time and orbital shaker driven agitation of 40 minutes and 120 cycles per minute respectively. The human blood studies went into considerable detail with regard to the overall biocompatibility of the HemoSep device, however, a summary of the results of the cell processing performance is shown in figure 4.9.

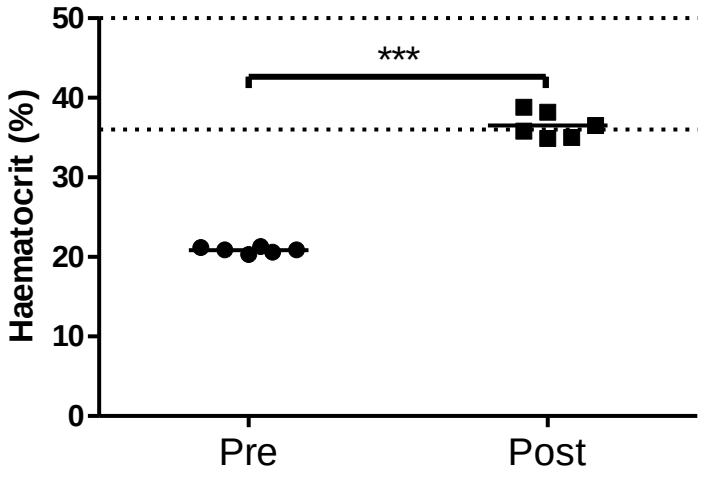


Figure 4.9 Rise in Hematochrit (PCV) in human blood over the 40 minute processing time. In all cases the PCV increased from 20% to within normal limits (36-50%). Data taken from National Blood Service Report. These data, taken from the study of human blood match very well with that derived from bovine studies.

A similar outcome was apparent in the analysis of platelet survival rates when the HemoSep device was employed with human blood (Figure 4.10).

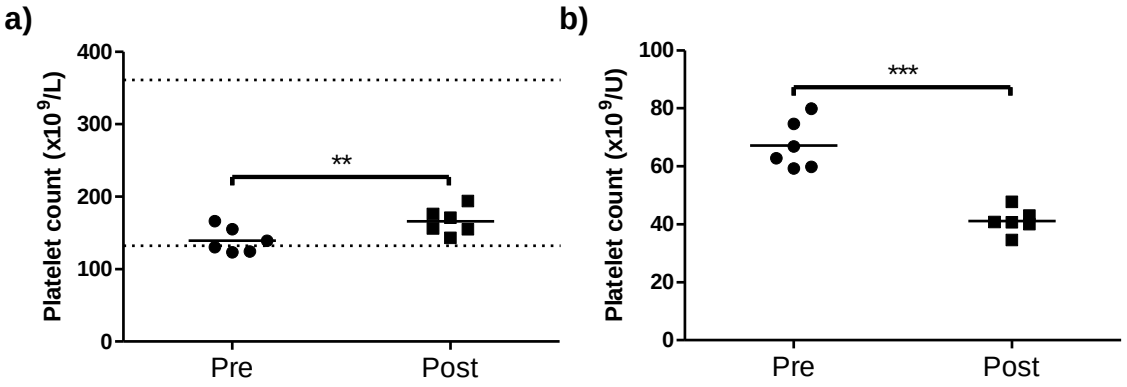


Figure 4.10 Analysis of effect of blood processing with the HemoSep device on platelet numbers. Figure 4.10a demonstrates that the device raises platelet numbers from sub-normal levels to within the normal range over the 40 minutes period. Figure 4.10b, platelet count per blood unit, shows that there is a net loss of platelets during processing, but this does not impact upon the normalisation of the platelet count associated with processing.

4.3 HemoSep and autologous fat transplant

The basic demands met by the HemoSep device in the whole blood setting are largely common to autologous fat transplant. The technology will focus on resolving the complex relationship between filtration media structure and superabsorber material.

A device for separating components of fluid comprising a container have least one separation or filtration member capable of selectively separating or filtering at least one component from the fluid, and a superabsorbent material capable of absorbing the at least component separated from the fluid, and thereby proving a processed fluid free or substantially free of the said at least one component.

The fat concentration device should ideally concentrate lipocytes whilst removing other elements, both cellular and in liquid form from the process maternal. To this end the experience with HemoSep suggests that the control membrane should have a porosity which closely matches the smallest cell size which is to be retained. In short, the larger the pore size, the faster the process will be. For blood processing where platelets of around 2-5 μm are to be retained, a 1-2 μm pore is required. For fat harvesting, where small cell species are not required, a much larger control pore size can be employed. The cell species of interest in this application, the lipocyte, is typically 70-100 μm (Table 4.1) in diameter. Therefore a control membrane with pore size of around 50 μm should produce a lipocyte rich produce. All other cell type are considerably smaller than 50 μm and will pass into the superadsorber with the carrier liquid.

CELL	UNITS
RBC	12-15μm
WBC	10-20 μm
PLA	3-5 μm
Lipocyte	70-10 μm

Table 4.1 Showing size range of blood cells and lipocytes.

Chapter 5 - Development of a novel superadsorber based autologous fat transfer technology

5.1 Design rationale

This project will focus on in the development of a novel fat concentration device based upon the HemaSep blood cell separation technology. Passive, superadsorbent driven, cell separation mechanics will be utilised to separate fat tissue from clinical liposuction waste material. This technology should be capable of separating lipocytes from the complex liposuction matrix. The study will focus on resolving the complex relationship between filtration media structure and superadsorber materials to maximise the efficiency of the passive separation process and to minimise any associated cellular disruption. A novel delivery device will be developed which will largely eliminate the need for manual manipulation and disruptive centrifugation, by utilising the combination of controlled filtration media and superadsorber technology.

5.2 Design considerations

Large volume of fat / oil / fluid material is available as a result of liposuction procedures. However, only small quantities of processed fat are typically used for reconstructive procedures. Therefore, rather than developing a device capable of processing the liters of liposuction material, we took the position that smaller volumes which are much easier to handle would be the focus of the present development. Under normal conditions around 120 ml of lipo-material should in a harvest of approximately 50 ml of injectable fat. In order that the device is a truly single vessel

solution to fat concentration, a syringe for reinjection should be incorporated into the system. The design flow therefore is as follows.

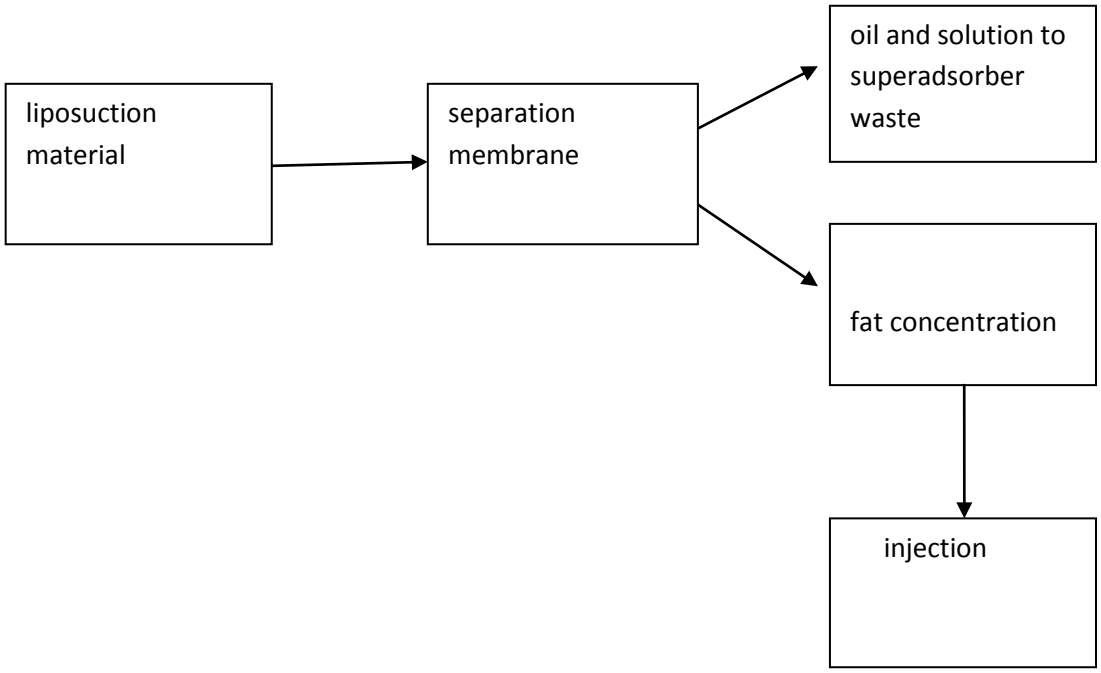


Figure 5.1 The diagram show the procedure of our design consideration.

To prevent tissue handling or transfer between process steps, a syringe based system with both a concentrate and waste reservoir is the elegant design solution.

5.3 Initial Technology Profile

To meet or design brief have designed a fat processing which removes the liquid element of the liposuction material using a membrane controlled superadsorer process device (Figure 5.2). This will provide a fat product which is ready for injection. The

entire process will take place in one device which incorporates the holding chamber, an interface locking mechanism, an adsorption control membrane and the superadsorber material (Figure 5.3 and Figure 5.4).

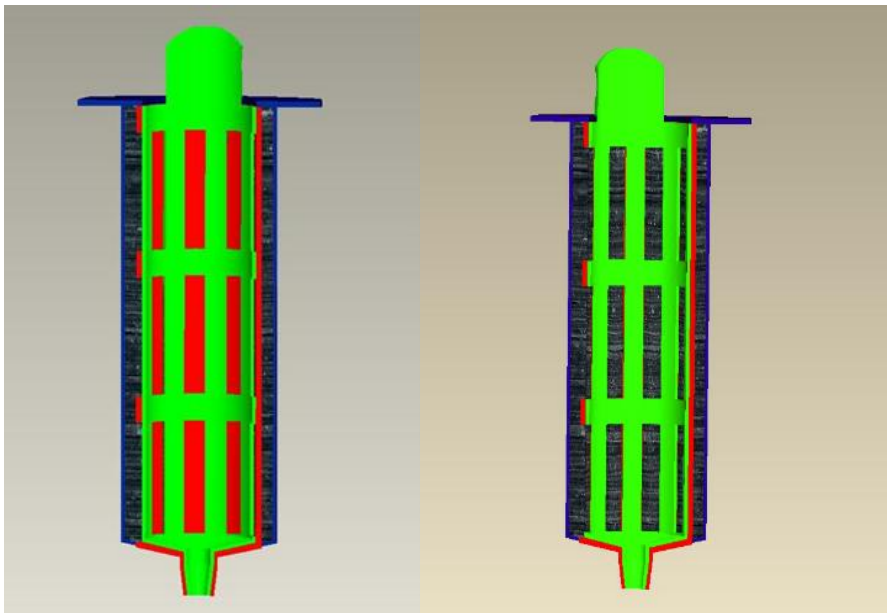


Figure 5.2 A cross-sectional view of the autologous fat separation device.

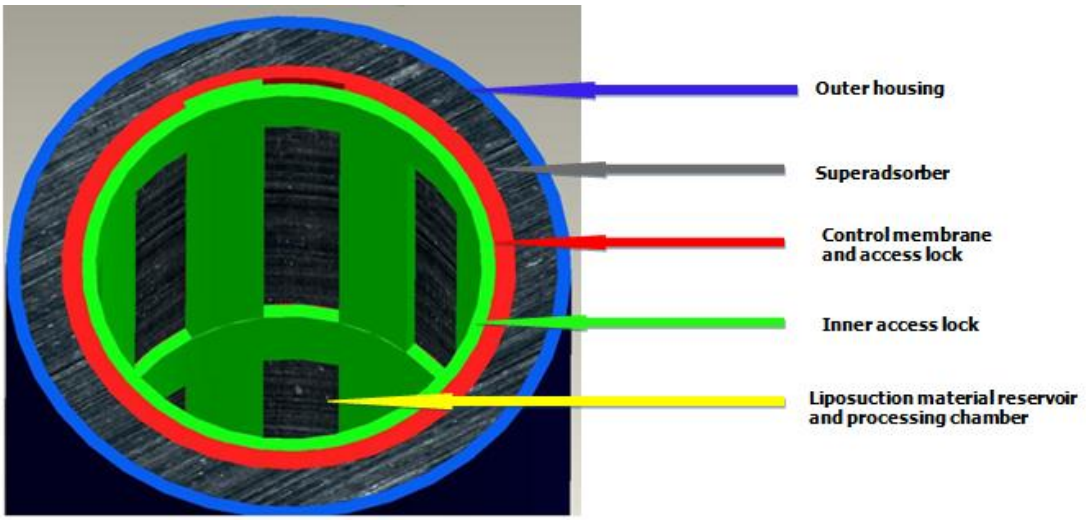


Figure 5.3 Showing device in the open-access position which permits interaction between the liposuction material and the superadsorber.

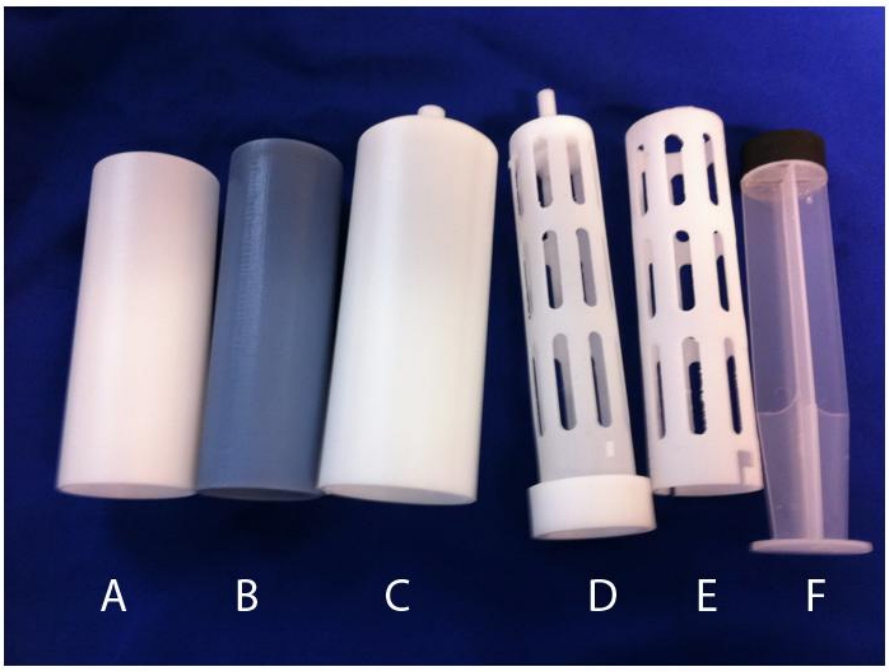


Figure 5.4 showing the component of the fat processing device.(A,B): chamber blanks. (C):outer chamber. (D):the chamber interface locker. (E):the chamber separater and membrane holder. (F): syringe plunger.

Key to the function of this device is the control membrane which separates the fluid chamber from the superadsorber chamber, and the superadsorber itself. In the present device we employed the same 2 um pore size polycarbonate control membrane as utilised in the HemoSep device and 12 grams of superadsorber, sufficient to adsorb up to 3 litres of liquid. Once concentration has taken place the inner separation column is rotated, blocking the connecting between the fluid and superadsorber (Figure 5.5). This closed chamber is then essentially a syringe barrel which can be empted using the syringe plunger. In this manner manual handling of the fat product can be avoided. Figure 5.6 show the function of superadsorber and filtration media controlled device.

Figure 5.5 The device connect with three way tap is ready for using, which can prevent liquid leaking out.

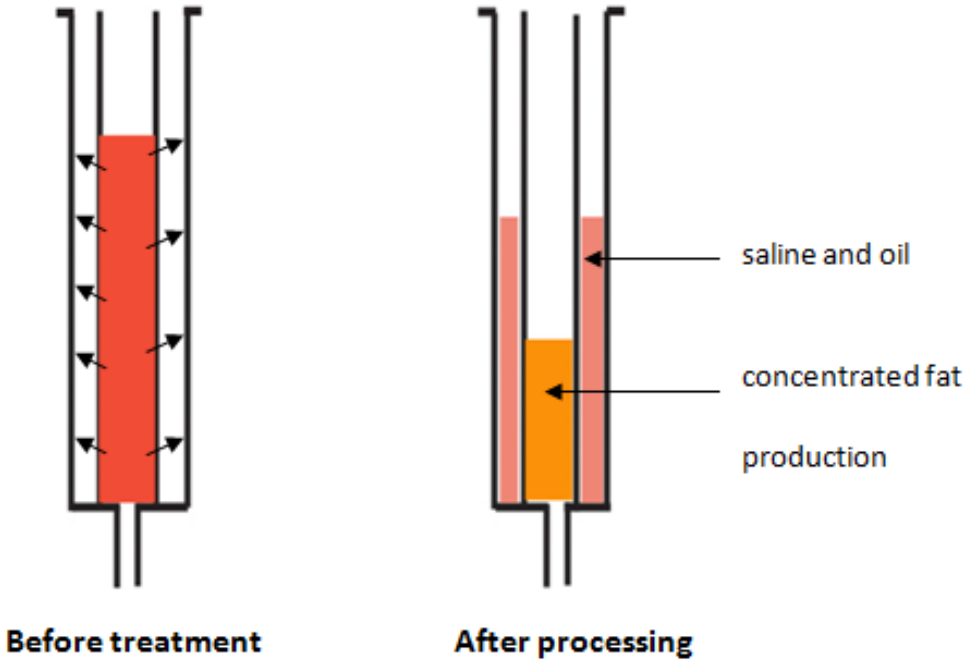


Figure 5.6 The function of the device. showing the fat sample are processed within the device compared to before treatment.

Chapter 6 Testing of the autologous fat transplantation device

6.1 Apparatus

Liposuction was performed using the apparatus showing Figure 6.1. This consisted of a SAM 12 variable vacuum system (MG Electric (Colchester) Ltd., Colchester, United Kingdom) and reservoir, PVC tubing connecting the SAM system to the cannula and the suction cannula itself. Figure 6.2 show the special designed cannuld for this study. For the purposes of the present work, a vacuum level of 750 mmHg was employed. The lipo-cannulae were custom built from stainless steel tube of 7 mm diameter and 350 mm length.

Figure 6.1 The liposuction device is prepared and ready for using.

Figure 6.2 The specially manufactured cannula was used in this study to harvest fatty tissues.

6.2 The liposuction procedure

The liposuction procedure was performed on the hind quarters of pigs supplied the abattoir on the day of slaughter.

On arrival in the laboratory the carcasses where raised to 38 ℃ by sealed immersion in warm H_2O. Saline solution was injected subdermally using a 50 cc syringe and large bore needle, in a manner similar to that carried out in clinical practice. The tissues were allowed to rest for around 30 minutes prior to initiating the liposuction procedure.

During liposuction a vacuum level of 60 mmHg was employed with the cannula being moved across the subdermal region until 1000 ml of tissue/ saline mix was collected in the collection reservoir. At this point the liposuction procedure was terminated and sample processing initiated. Figure 6.3 show the liposuction in process.

Figure 6.3 Aspiratiing the fatty tissue from the pig leg.

6.3 Sample processing and analysis

A number of factors were analyzed in assessing the effective or this approach including

I) The concentration change associated with processing the fat containing material compared to control (unprocessed) levels.

II) The quality of the fat concentrates as expressed by the prescense of intact adipocytes after processing.

III) The processing time required to reach appropriate concentration levels.

To assess these factors, samples were taken from the initial liposuction pool, as control samples. In doing this the adipose/saline mix in the collection chamber was continuously agitated and samples taken from the mid-level to ensure that a standard "mix" or material was harvested for analysis.

6.4 Identification of intact adipocytes

Oil red O stains (OROS) was utilized to stain the adipose cells in the tissue samples. This stain is a unique lysochrome which is predominantly used to indicate triglycerides and other form of lipids, sometimes, it also stains some protein-bound lipids. Oil red O stain has been utilized in previous adipose cell studies (Gonzalez, et al, 2005; Gourlay, et al, 2006; Park,et al, 2008) and stains a red or deep red or orange colour that is highly visible under appropriate light microcopy conditions. The standard procedure of staining is that, 2 ml of gently mixed adipose tissue is place into a test tube, 200 μl of Oil red O stain working solution is added, and then, each sample is mixed by a vortex mixer for approximately 1 minute, incubated for 30 minutes at room temperature. Following this samples were taken and introduced into improved Nehabauer Hemocytometer chambers. The samples are examined using simple light electronic microcopy utilizing low (×10) high (×40) power, and the status of any adipocytes present recalled and reported. The adipocytes where recognize principally by size and structure.

6.5 Measurement of fat concentration

The fat suspension taken as a result of liposuction was introduced into the processing

device, after the level of fat present was measured by centrifugation. The solution was processed by the device, using gentle orbital shaking, for 30 minutes in one group (N=7) and for 60 minutes in a second group (N=7). Samples of the fat solution were harvested post-processing for assessment of the level of fat concentration. The percentage fat present was assessed by visual inspection and measurement.

6.6 The adsorption capacity of the device

The fat processing device is restricted in its adsorption capacity by two factors:

(a) The adsorptive capacity of the superadsorber material

(b) The volume of the collection chamber

Studies carried out as part of present project, by titrating water onto a 1 g sample of superadsorber has confirmed that it is capable of adsorbing around 350ml/g before reaching saturation (Figure 6.4).

Figure 6.4 The capability of the suporbabsorb we used for our novel device which can aspirate approximately 350 ml liquid.

This should be sufficient to fully saturate the adsorption chamber which has capacity calculated to be in the region of 89.4 ml. (see calculation below). The capability of the device is shown in Figure 6.5.

Volume of adsorption chamber = volume of outer chamber - volume inner chamber

$VADS = (\pi r^2 x\ L)^{outer} - (\pi r^2 x\ L)^{inner}$

$= 217.7 - 128.229$

$=89.4$ ml

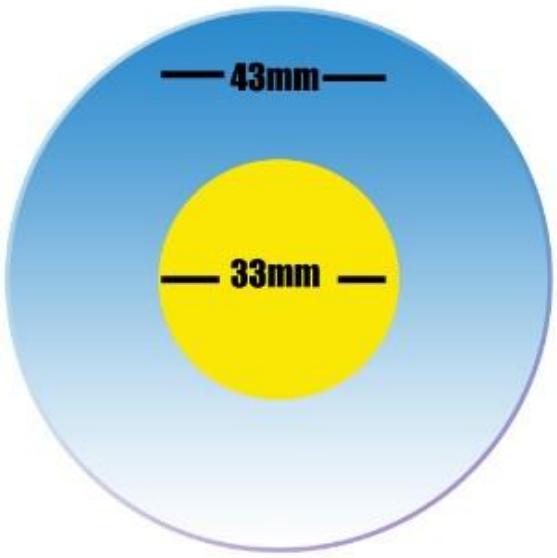


Figure 6.5 Showing the outside and inside diameter of the device.

To achieve a fat concentration of 100% after processing of 128.3 ml of fat / fluid mixture, the input concentration of fat for saturation of processing in chamber to be reached would require a fat concentration input solution of the order of 30.3 %. Any lower concentration of fat in the processing solution would result in sub-optional processing and resultant fat concentration of less than 100%. Fat concentration at the

start of processing in excess of 31% represents the best possible situation for reaching adequacy in fat concentration after processing.

6.7 Processing time

The time taken to process the fatty solution to acceptable levels is a key element of this story. Therefore, buildings on the experience with HemoSep, to processing times were investigated, 30 minutes and 60 minutes. These were carried out in two groups of 7 experiments for comparative purposes. All experiments followed a common protocol, with the exception of the processing time.

6.8 Statistics analysis

Where appropriate, due the minimal complexity of the date, Student's t-test was employed to compare statistical significance of outcomes with a p-value of less than 0.05 signifying significance.

Chapter-7 Results

7.1 Fat concentration

Around 1 litre of fat / saline suspension was recovered from each procedure. 120 ml samples of this material were taken for processing with the new fat concentration device. Prior to processing the level of fat present was determined by centrifugation (Table 7.1).

Procedure Number	%Fat	Mean+SD
1	6	
2	6	
3	8	
4	10	8.0±2.30
5	11	
6	5	
7	10	

Table 7.1 The result of the samples by concentration measurement after 30 minutes which show the concentration rate is low.

Other group procedures were carried out over 60 minutes and resulted in increasing fat concentrations to 7% (Table 7.2).

Procedure Number	%Fat	Mean+SD
8	7	
9	7	
10	10	
11	10	8.71±2.4
12	12	
13	5	
14	10	

Table 7.2 The result of fat processed by concentration within 60 minutes.

The objective of this study was to develop a device which is capable of concentrating the fat product to around 85% for cosmetic injection. The concentration level after 30 minutes of processing fell well below this, reaching only 29±4.16% (Table 7.3). However, this level was reached from a very low baseline of only 8.0±2.3%, representing a rise of around 21%.

Procedure Number	Post-processing volume fat	Mean+SD
1	23	
2	26	
3	32	
4	32	29±4.16
5	27	
6	28	
7	35	

Table 7.3 The results of fat processed by concentration measurement after 30 minutes within the device.

7.2 Analysis of fat concentrate

Sample of the fat products were taken after processing to determine whether intact lipocytes where present in the concentrate or whether total cellular disruption is the result of this process. Typical light microscopy images of Oil red O stained slides of lipocytes from these studies are shown in Figure 7.1.

These show quite clearly a significant number of intact lipocytes in the samples, suggesting that the process is, as anticipated, sparing of cellular material. The lipocytes were identified by general morphology, together with ORO stains and size.

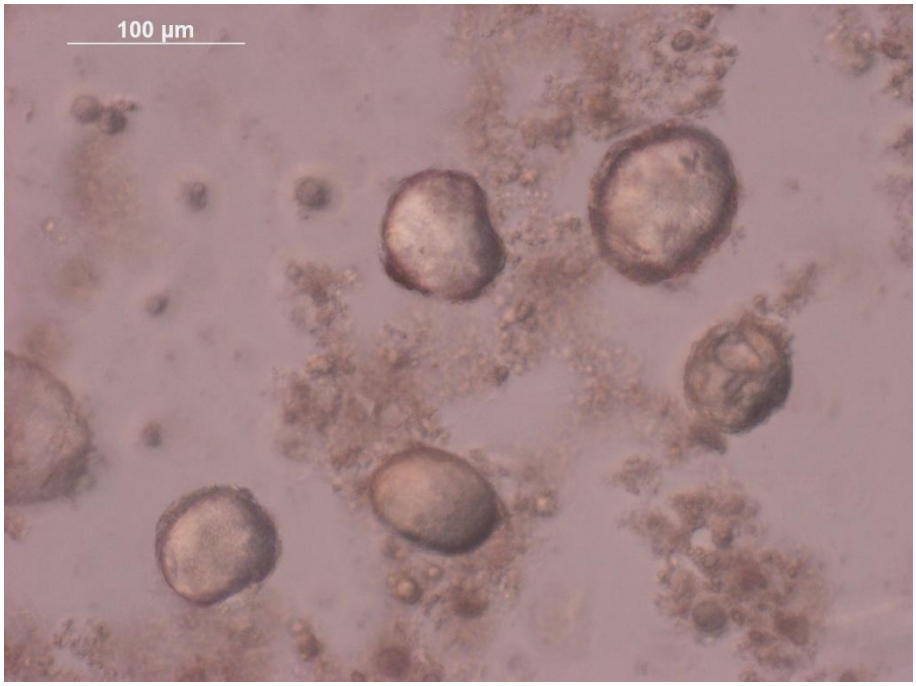


Figure 7.1 Intact adipocytes were observed by light microscopy (×10) after fat processing within our device.

7.3 Processing time

The objective of this project was to develop a "rapid" fat transfusion product. Therefore, in keeping with normal clinical practice a 30 minutes process time was the target. This falls within the processing envelope of the HemoSep device and, it was thought should be achievable. However, to establish the effect of processing time on performance two timelines were studies: 30 minutes and 60 minutes, one representing the ideal clinical processing speed and the other the extreme of acceptable processing time. Apart from processing time all protocol parameters were common. The results of the 60 minutes processing time can be seen in Table 7.4. The additional 30 minutes

processing time resulted in a statistically insignificant ($p < 0.05$) increase in concentration after the initial 30 minutes processing time, form 29±4% to 31.4±3.77%.

Procedure Number	Post-processing volume fat	Mean+SD
8	25	
9	30	
10	35	
11	35	31.42±3.77
12	30	
13	30	
14	35	

Table 7.4 Fat samples process within this device after 60 minutes indicate the fat product volume is the same as at 30 minutes processing.

7.4 Initial review of results

The study demonstrated that the new fat concentration device functioned in two key parameters,

(I): It demonstrated fat concentration

(II): It was associated with preservation of lipocytes

However, it is clear that the device was not capable of achieving target concentration values of around 85%. This may be due to a number of factors, but most likely and

easily addressed of these, is the restrictive volume of adsorption chamber. It would appear that the adsorption capacity of the system was reached at the 25% - 35 % concentration level. As the 12 g of adsorption material in the system is capable of adsorbing in excess of 12 L of fluid, the observed limitation to adsorption must be due to other factors. The restrictive 89 ml volume of the adsorption chamber may be responsible for this. This is supported by the factor that extending the processing time had no effect on the level of fat concentration. Further evidence of this was apparent from the first thesis of experiments where the superadsorber leaked from the chamber to the exterior of the device (Figure 7.2), suggesting a need for further space for volume displacement. These data supported further development of the device to incorporate a larger adsorption chamber (Figure 7.3). A new configuration was therefore design with an adsorption chamber capable of adsorbing 210.9 ml of fluid. This device was further tested using the protocols employed for the previous iteration over 30 and 60 minutes.

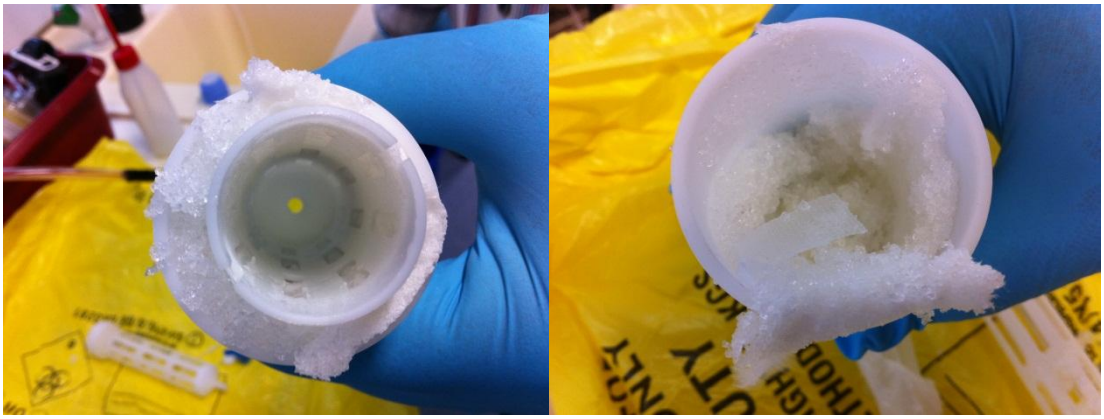


Figure 7.2 The saturated superadsorber can be seen in this figure. Critically, the superadsorber studies adsorbed after 30 minutes due to expanshion restructions associated with the limited volume of the collection chamber.

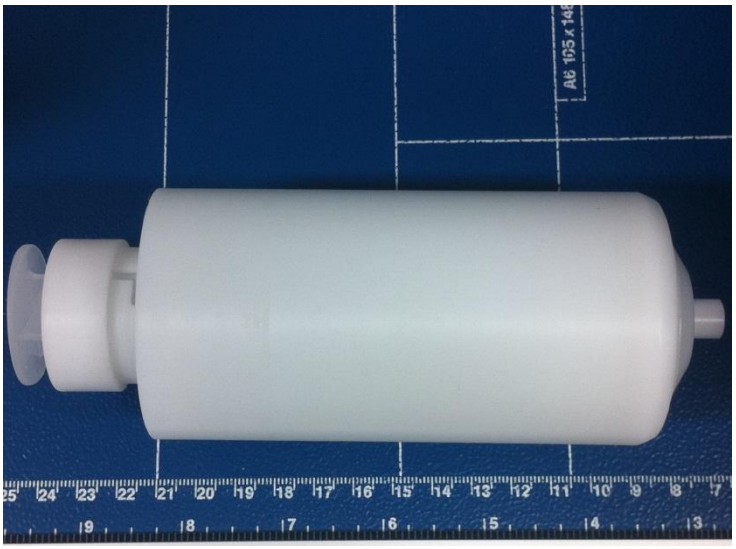


Figure 7.3. The secondary designed device which with additional volume of adsorption chamber.

7.5 Results of 2nd iteration testing

The new device, with a larger absorption chamber was found to produce more acceptable results (Table 7.5).

Run Number	Pre –Fat % Mean±SD	Post-Fat% (30 min) Mean±SD	Post-Fat% (60 min) Mean±SD
1	5	62	68
2	6	78	81
3	5	83	84
4	10	68	70
5	11	87	86
6	6	78	81
7	6	67	67
	7.12±2.5	74.71±9.2	79.14±7.17

Table 7.5 Fat process after 30 minutes and 60 minutes within the device of secondary made.

These data suggest that there is little adsorption beyond 30 minutes with fat concentration rising from 74.71±9.2 to 79.14±7.17 (Table 7.6), during the last 30 minutes of the test run ($p>0.05$). Indeed, although not measured as part of this study, peak concentration levels were observed to be reached within the first 30 minutes, reflecting the experience with the HemoSep device in concentrating blood. However, it is clear that these data represent a significant improvement upon the previous

version of the device. The difference concentration levels reached by the two versions of the device are shown in table 9, at 30 and 60 minutes processing periods.

Iteration	Time	Concentration	P
1	30	29±4.16	<0.001
2	30	74.71±9.2	<0.001
1	60	31.42±3.77	<0.001
2	60	79.14±7.17	<0.001

Table 7.6 The different concentration levels by the two version device after 30 minutes and 60 minutes processing respectively.

The morphology and quantity of adipocytes with the novel and advanced devices shows an elevated number of fatty cell cluster (Figure 7.4), and a great majority of intact adipocytes (Figure 7.5).

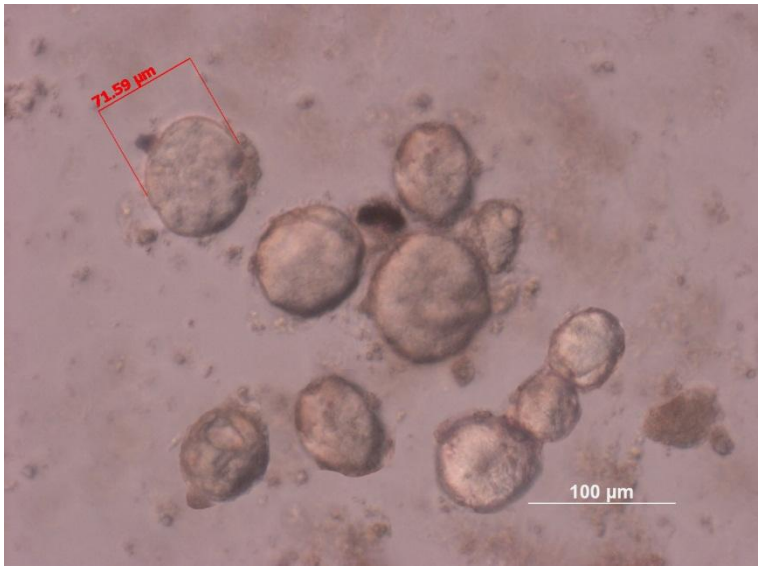


Figure 7.4 Intact adipocytes were observed by light microscopy after fat processing within our device (×20).

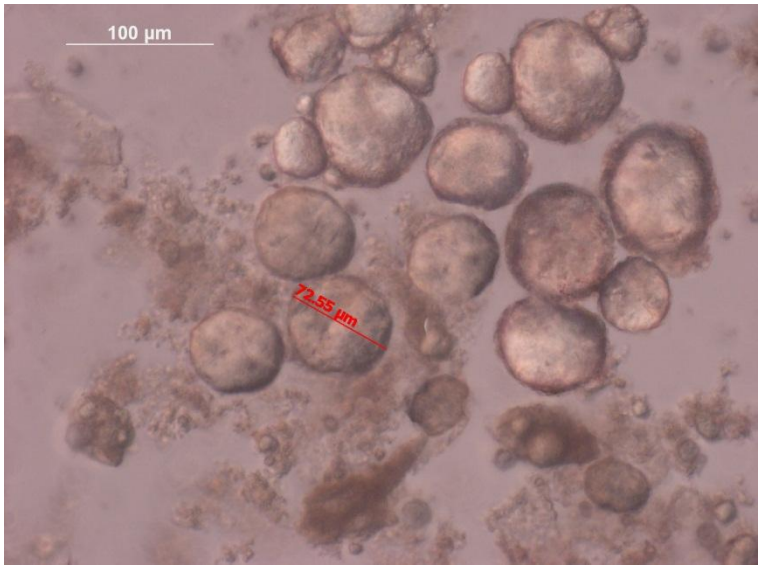


Figure 7.5 Light microscopy photographs show the clusters of adipocytes (×10).

Chapter-8 Discussion

Autologous fat transplant for recontouring of tissue defects and augmentation has a history going back to Neuber in 1893 (Neuber, 1893), Bircoll was the first to use liposuction derived autologous fat grafts to fill defects (Bircoll, 1982). Since then AFT has been widely utilized, and a large number of different well-described methods for autologous fat transplantation in aesthetic plastic and reconstructive surgery has evolved. Nevertheless, thus far, there is no universal agreement as an ideal technique to process the adipose tissue before injection. There is however some consensus that a simple inexpensive, single step process which does not require multiple tissue handling steps would be ideal.

Desorption rates of autologous fat graft have been attributed to trauma of harvest and the quality of the autologous fat process. Many authors recommend that using half general suction pressure and processing adipocytes through an optimal technique to avoid fatty tissue mechanical injury. Nguyen (1990) described that 90% adipocytes were injured at 760 mmHg on histological examination, in contrast, only 5% adipocytes injury after gentle aspiration. This work highlights the importance of the tissue processing to the success of the grafting procedure.

In an attempt to meet the clinical need, we created a novel device and focused our study to meet modern clinical practice in term of the harvest and process techniques (Katz & Arnold, 2010; Kaufman, et al, 2006; Fouriner, 2000; Markey &Glogau, 2000). Autologous fatty tissue was harvested by lipoaspiration, and placed into our

device which both filtrated and concentrated the tissue suspension using a control membrane, superadsorber and orbital shaker. During this procedure 128 ml of fat tissue suspension can be processed within our sterile and closed device which can remove blood and fluids, a process which is known to decrease the resorption rates of injected fat tissue and to maximize the longevity of clinical results. Under the laboratory conditions the entire process takes approximately 30 minutes.

A number of critical process factors become clear during this study, some of which resulted in a re-design of the device to improve the performance of the technology. Our early studies with the first iteration of the device were only moderately successful, but were associated with fat concentration. However, the level of concentration over 30 minutes (our target process time) was somewhat less than expected, reaching only 29%±10%, albeit from a very dilute starting point of 7.3%±3.1%. This level of concentration is not suitable for clinical use, and further studies were carried out using longer processing time of 60 minutes. This extended processing time did not deliver better performance, resulting in concentration levels only slightly, but not statistically significance higher, 31.7%±7.3%. Clearly there was an issue with performance which was not time dependant. Our experience with the HemoSep technology suggests that there was adequate membrane surface and superadsorber present to achieve much better performance than was apparent, therefore, the restriction must lie elsewhere. The issue of the volume of the adsorption chamber was addressed in a second iteration of the device, giving the superadsorber more room to expand as it adsorbs fluid. The superadsorber chamber was increases in capacity from 89.4 ml to 210.9 ml, and 30 minutes and 60 minutes studies repeated. These were much more successful delivering concentrations of 74.71% ± 9.2%. after 30 minutes and 79.14% ± 7.17%

after 60 minutes. These data suggest that clinical quality natural could be produced with the new device after 30 minutes.

The quality and survival rate of autologous fat graft is improved. According to Peer`s theory of cell survival, if the autologous fat graft possess intact and higher number of adipocytes, the fat graft survival will be better enhance (Peer, 1955). We subsequently evaluated the size and morphology of fat cells present in our concentrate. Our results from the present study illustrated that the autologous fat graft contain considerable number of viable adiposcytes with our new device. This is a major advantage as it appears to protect cells, maintaining intact histologic structure and viability of lipoaspirated fatty tissue. These results indicate that our novel processing technique represents an efficient method for concentration of fatty tissue. Its gentle action combined with of controlled filtration media and superabsorption technology are key to this. Recent studies resulted in a high quality fat product (Krulig, 1987; Lewis, 1992) described the presence of blood in prepared autologous fat graft and the role of this in stimulating macrophage to remove adipocytes. Our device is designed to remove blood products, leaving only fat concentrate for re-injection. This may be a key clinical advantage of this technology, but requires further study.

Other research (Chajchir & Benzaquen, 1989) showed that the diameter of cannula probably affects on the quality and longevity of adipocytes. While others claimed that diameter size does not influent the result .The present study was carried out under laboratory conditions on isolated pig hind limbs, therefore issues which influence the quality of the fat concentrate other than the process itself could not be investigated.

Chapter 9 - Conclusion

The results of the present study demonstrate that this novel approach to the preparation of fat graft tissue for plastic and reconstructive surgery works very well. The level of fat concentrate delivered as a result of the second iteration of the device was of near-clinical quality. The process would appear to spare adipocytes and remove all fluid from the input suspension. Some future consideration must be made with regard to the control membrane configuration, possible increasing the pore size to 20 microns, which will ensure adsorption of blood products and increase process speed.

Chapter - 10 Further work

The work offers much promise in terms of the development of an integrated solution for fat harvesting for reconstructive procedures. However the results suggest that there may be some way to go in relation to optimizing the approach. In particular, future work will focus on：

1) Further testing of membrane pore size efficiency to determine the most suitable membrane material and pore distribution for this application. The current work suggests that larger pores may be possible, and that this will positively impact upon processing time. In addition the use of materials with different zeta potential may offer the possibility to reduce fouling of the membrane during use, further enhancing the process time.

2) More detailed histological assessment of fat concentrate. The present work only touched upon histological examination of the aspirate. However, further study is required to ensure that the cells processed are viable and are not contaminated by unwanted cell species. The objective is to deliver, if at all possible, a pure fat product to recipient’s patients and further investigation of the aspirate will enable better processing.

3) Use of device on human liposuction suspension without reinjection. This is the next step towards clinical delivery of the device and will ensure that there is a correlation between the results of the animal studies and that experienced through processing human tissue.

4) Regulatory review. The device requires a full regulatory assessment prior to commercialization and clinical study. Although on the face of it, the device appears to comply with a category II device; this needs further investigation and regulatory advice.

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Development of a Fat Concentration Device for Plastic and Reconstructive Surgery Applications

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A Thesis Submitted for the Degree of Master of Philosophy in Bioengineering

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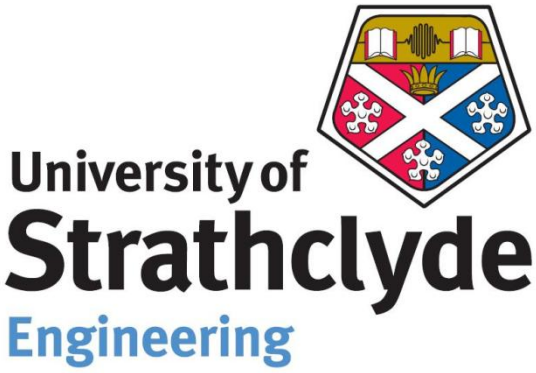

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Declaration

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed…….… Date……..

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