Use of Microcannula Closed Syringe System for Safe and Effective Lipoaspiration and Small Volume Autologous Fat Grafting

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Introduction: The purpose of this study was to provide background on methods of acquiring autologous adipose tissue as a tissue graft and a source of adult progenitor cells for use in cosmetic plastic surgery; to discuss the background and mechanisms of action of closed syringe vacuum lipoaspiration, with emphasis on accessing adipose tissues for use in aesthetic, structural reconstruction and regenerative applications; and to explain a proven protocol for acquiring high-quality autologous fat grafts with use of closed syringe, disposable, microcannula systems.

Materials and Methods: An explanation is provided for the components of and the advantages of using the super Luer-lock and microcannulas system with standard Luer syringes. Equipment selection is described, and a sequential explanation is presented for conducting minimally traumatic lipoaspiration in small volumes, including use of blunt injection cannulas to reduce risk of embolism.

Results: Thousands of autologous fat grafts have been used in cosmetic surgery and have proven safe and efficacious for use as part of liposuction techniques for large and small structural fat-grafting procedures. The importance and advantages of gently harvesting the adipose tissue complex has become very clear in the past 5 years. A closed syringe system offers a minimally invasive, gentle system to mobilize subdermal fat tissues in a suspension form. Resulting total nuclear counts suggest that this approach achieves higher yields than use of applied vacuum systems that are always on and use a constant mechanical pump.

Conclusions: Use of disposable closed syringe lipoaspiration systems featuring disposable microcannulas offers a safe and effective means of harvesting small volumes (<100 mL) of nonmanipulated adipose tissues and accompanying progenitor cells within the adipose-derived stromal vascular fraction. This article presents a practical step-by-step protocol for acquiring high-quality autologous fat grafts.

For many years, cosmetic plastic surgeons have recognized the value of low-pressure liposuction for successful transplantation of adipose tissue for structural augmentation. In the introductory years (1980–1990) of liposuction techniques, autologous fat grafting was considered unpredictable. Once bioengineers discovered the actual mechanisms by which lipoaspiration worked, the closed syringe system for gentle harvesting and transplantation was developed and patented. Early belief that effective lipoaspiration was directly related to the force of the vacuum was replaced by the understanding that the introduction of fluid into the fat layers permits the adipocyte cells and stromal elements to enter into a suspension state. This suspension is then easily extracted through the use of closed syringes, thus providing adipose tissues with reduced cellular damage and leading to improved and more predictable grafting results.

As the importance of tumescent fluid distribution was appreciated, more value was placed in extensive pre-tunneling (ie, moving the cannula without applying vacuum). This better distributes the local solution and enhances the ability to mobilize the adipose tissues into a suspension state, yielding more successful structural grafting and predictable autologous fat graft (AFG) results.

In the early 2000s, appreciation of the potentials of adipose tissue and its related stromal elements led researchers to examine the adipose-derived adult stem-stromal cell content within the adipose tissue complex. Evidence has shown the importance of these associated nucleated cells as integral contributors to
the tissue maintenance and healing processes. Studies of adipocyte homeostatic replenishment (following normal senescence and cellular death) show that these attached cells within the adipose complex are activated to create adipocytes and thereby maintain adipose tissue integrity over time. As safe, plentiful, and easily accessible graft tissue is provided by gentle lipoaspiration, utilization of the entire adipose tissue complex as a central focus in optimizing effectiveness of autologous fat grafting in cosmetic plastic surgery and clinical regenerative medicine has evolved.

Preclinical and clinical applications have been reported in many scientific studies in the biological, bioengineering, and clinical medical literature. Cosmetic plastic surgeons initially focused on understanding the mechanisms to achieve safe and effective AFGs. It was believed that intact cellular (mature adipocytes) transplantation was the most important goal. However, it is now understood that the mature adult adipocytes transplanted may be the least important feature producing long-term success, even in structural fat augmentation graft applications. Current beliefs are that success with long-term AFGs is actually due to activation of associated cells, followed by proliferation of those cells to differentiate into the adipocytes for volume replacement.

For example, placement of lipoaspirants into existing adipose tissue favors proliferation and differentiation into adipose cell phenotypes. As understanding of the maintenance (homeostatic) and replenishment of adipose cell cycles in vivo increases, extensive research is being devoted to the study of the microenvironment (niche), cell-to-cell/cell-to-matrix factors, and autocrine/paracrine signaling system functions.

Rapidly accumulating clinical data on the safety and efficacy of autologous fat grafting provides clear evidence that fat tissue grafts possess extensive potential in wound healing, well beyond the structural augmentation in cosmetic plastic surgical uses.

Understanding these mechanisms has resulted in important application potentials for aesthetic, reconstructive, and regenerative medicine. Preclinical, early clinical, and controlled studies in animal and human models show worldwide recognition of the potential uses of these cells in diverse areas of medicine and surgery.

AFGs for Use in Aesthetic Reconstructive Surgery

Research and clinical applications have led to an appreciation of the existence of a large population of heterogeneous nucleated cells and extensive native bioactive scaffolding that is an integral component of adipose tissues (Figure 1). In addition, advanced understanding of the processes of homeostatic adipose replacement has shown that carefully harvested autologous fat grafting for structural augmentation is effective and has become much more predictable and understood than in years past. Though it was once thought that adipocytes do not cell divide, it is now clear that adipose cells turn over at a rate of replacement every 5–10 years. The initial steps in that turnover involve natural senescence, wherein a waning mature adipocyte secretes specific growth factors and signal proteins, which act via autocrine and paracrine actions on the adipose niche. With activation of attached, near terminally differentiated precursor cells (preadipocytes) respond by activation and entrance into a metabolic state. With accumulation of lipid droplets and active metabolic activities, the lost mature cells are replaced by young adipocytes.

The closed syringe system, with its array of microcannulas (small volumes <100 mL) and standard volume reduction cannulas (for >100 mL lipoeduction and contouring), is a recognized and proven lipoaspiration system. Explanation and a detailed discussion of a repeatable, effective, and safe protocol for adipose tissue complex harvest for cosmetic plastic surgeons will be provided in this article.

Materials and Methods

Selection of Lipoaspiration Sites

The lower abdomen and flank areas of males and females are considered ideal sites because of the distribution of human adipose tissues and relatively large

Figure 1. Adipose tissue complex native scaffold (decellularized, lyophilized adipose tissue showing native 3-dimensional matrix).
deposits. Aspiration sites in the medial and lateral thigh/buttocks areas are sometimes favored for lipoaspiration and adipose graft harvesting in female patients because of genetic distribution within the gynoid body type. In patients with a very low percentage of body fat who need autologous grafts, use of a high-definition ultrasound probe is helpful for determining the thickness and depth of adipose deposits that can be acquired.

Preparation of Lipoaspiration Sites (Donor and Recipient)

The patient may be placed in the supine or lateral decubitus position to facilitate the preparation and complete sterile isolation of the proposed donor area(s). It is important to follow a standard sterile protocol for the harvesting and placement sites. Routine operative-site asepsis should be maintained in all cases, and patients should be marked in an upright or standing position to effectively identify the area of available or unwanted adipose tissue deposits.

Microcannula Instrumentation

The patented Tulip Medical (San Diego, Calif) closed syringe system for lipoaspiration features very smooth cannulas and a super Luer-lock (Tulip Medical) connection for use with standard Luer-lock syringes (Figure 2). This hub connection is a very important component of the closed syringe and microcannula system in that it provides an excellent seal for maintaining the even vacuum forces that are desirable during lipoaspiration. As the super Luer-lock connection seals at both the internal Luer connection and the outer ring of the standard Luer connection (Figure 3), it thereby provides a very stable, rigid base, which is desirable when using very small cannulas (and dealing with their associated flexibility) within the tissues.

Internal female Luer-type connectors on some microcannula systems become less efficient when cannulas are redirected, placing a torque on the junction of cannula-syringe barrel and allowing air leakage into the closed system (particularly in the longer cannula selections). This does not usually prevent aspiration capabilities, but it does decrease the efficiency and may introduce cavitation to the harvest tissues.

Two standard options for microcannula selection offered within the Tulip system are the Cell-Friendly autoclavable microcannula (Tulip Medical) (Figure 4) and a sterile, coated disposable microcannula (Figure 5).

CELL-FRIENDLY MICROCANNULA (AUTOCLAVABLE)

These cannulas are internally polished by a micro-abrasive extrusion process to maximize internal smoothness and reduce adipose tissue damage to the adipocytes, the precursor cells, and their accompanying matrix. External cannula anodizing processes provide a smoother surface for ease of passage within the subdermal adipose plane. This is a popular design used by plastic cosmetic surgeons for performing liporeduction and harvesting AFGs for structural augmentation procedures and larger-bore cannula sets. In cannulas <3.0 mm, it is important to thoroughly flush with a water-prep soap mix, followed by ultrasonic cleaning, and then thoroughly refush with water before steam or gas sterilization. It is very important to avoid use of brushes for internal cannula cleaning, as they will damage the highly polished interiors.
STERILE, COATED DISPOSABLE MICROCANNULA OPTION

Use of microcannulas in small diameters of less than 3.0 mm (range, 0.9–2.4 mm outside diameter [OD]) presents a significant challenge to ensure the proper and effective cleaning/sterilization cycles that are mandatory with reusable microcannulas, making a disposable option attractive, particularly in the smaller-diameter cannula group. The disposable products are packaged and labeled in a sterile wrap and can be opened directly onto the sterile field or the back table. Featuring the super Luer-lock base, these stainless steel cannulas are totally coated internally and externally with a lubricious material that provides an extremely smooth coating, permitting easy passage through adipose tissues with minimal resistance and trauma. Initially, hydrogel coatings were applied on the internal and external surfaces but have been replaced by more efficient and effective coating materials that are at least 20 times more lubricious than previous coating materials. It is believed that the less cellular and tissue trauma created, the better the quality of the adipose grafts. With increased recognition of the difficulties in effectively cleaning nondisposable microcannulas (<3.0 mm), most surgeons are choosing completely disposable infiltration, harvesting, and injection cannulas.

Selection of Microcannula Length and Diameters

For small-volume applications (<100 mL), it is recommended to use a small, multiport infiltrator cannula for even and thorough distribution of local anesthesia throughout the adipose donor layer. Openings near the tip are multiple and oriented for a 360° distribution of local anesthesia while moving through the subdermal fat layers. It is common for practitioners to use this infiltration cannula in diameters of 2.1 mm OD and a length of 10–20 cm (Figure 6).

Harvesting cannulas are designed to acquire the adipose tissue grafts from the subdermal fat plane, following the same pattern and location of local anesthesia distribution. The openings on the harvesting cannulas are typically in line or offset (meaning in a nonlinear pattern of openings near the tip of the cannula). These vary in diameter between 1.67 mm and 2.4 mm (OD) and are 10–20 cm long. Selection of a slightly shorter harvesting cannula, compared to the length of the infiltrator, makes it somewhat easier to remain within the local anesthesia distribution areas for awake patients.

Syringe locks come in two options, external and internal. The external locks are specifically designed for use on Becton Dickinson (Franklin Lakes, NJ) or Monoject 10/12 (Coviden, Mansfield, Mass) and 20 mL Luer-lock syringes and 60 mL Toomey tip syringes to hold the syringe plunger in a fully drawn position during the application of vacuum (Figure 7). Before application of vacuum by pulling the syringe plunger to the desired level, it is essential to draw sterile saline fluid into the cannula to completely displace all air within the system. When pulled and twisted into the locked position, the edge of the lock engages the side of the plunger, permitting the physician to apply even and gentle vacuum pressures while moving the cannula through the tumesced adipose layer. The internal-type lock is called a snap lock; these universally fit a variety of syringes and sizes from different manufacturers.

Anaerobic transfers (Luer-to-Luer) are available to facilitate near anaerobic loading of treatment syringes before grafting procedures and for optional use of additives to the grafts (eg, combining platelet concentrates, such as high-density platelet-rich plasma [HD PRP], to the adipose grafts) in the same syringe. They are also useful for transferring the graft treatment mix into syringe sizes of the physician’s preference for injection and for avoiding undesirable exposure of the harvested graft to air (Figure 8). It is important to avoid excessive air exposure to grafts because of the potential for contamination by airborne particles or pathogens. Techniques described as helpful in free lipid removal (such as Telfa [Coviden] rolling) are vulnerable to such contamination.

A controlled aliquot injector gun is available for placement of controlled 0.5-mL aliquots of graft into the prepared tunnels and locations. When additives are added to the adipose tissue graft, the density of the injection material may be increased. As a result, the physician may need to exert more force to inject into the tissue site or may encounter sudden and uneven distribution of desired small aliquots of graft with prepared tunnels associated with adipose matrix density within the graft itself. The single trigger pull provides exact volumes of solution to be placed with less pressure required by the provider (Figure 9).

Sequential Technique for Performing Microcannula Liposuction

It is recommended that the area of donor and recipient sites be outlined using a skin marking pencil with
patient in an upright position. This will become the area prepped/draped to expose the thickest deposit of palpable fat tissues and serve as a distribution pattern for local anesthetic infiltration (Figure 10).

After marking, preparation, and sterile isolation of the donor area, an 18–20 g needle, side edge positioned vertically, is used to create a small slit-like opening, extending through the epidermis and dermis into the subdermal fat plane of the donor site. It is important to avoid too large an opening, as the closed syringe system vacuum depends on maintaining a tight side-wall opening to ensure even vacuum application. Use of stab incisions with scalpel blades of number 15 or number 11 sizes tend to create an opening larger than necessary or desired. This opening is made larger by selectively cutting the dermal layer (under the skin surface) with the edge of the needle bevel. This allows the introduction of the multiport infiltration cannula through the skin and the subdermal fascia (and should perforate and remain below the Scarpa’s fascia in the abdomen) (Figure 11). In large cannula sizes, use of a tapered stainless sharp trocar (3 mm) is used to permit snug fit of the aspiration cannulas into the desired space.

After entry, the multiport infiltrator cannula is passed in a horizontal fashion within the subcutaneous donor fat deposit, above the muscular layer, in a spokes-of-a-wheel pattern. Pinching the skin-fat tissues may help pass the cannula. During movement of the infiltrating cannula, very slow injection of the tumescent local anesthesia fluid is provided on both the entry and withdrawal strokes, evenly and in layers. The importance of avoiding pooling of the local anesthetic is that evenly distributing liquids improves the efficiency of harvest because of the need to provide a suspensory fluid carrier for the adipose graft tissues and to ensure excellent patient comfort.

In typical small-volume grafting cases, local or tumescent solution ranging from 20–30 mL is used during the infiltration process, following a general guideline of at least a 1:1 ratio to anticipated fat harvest volume. For example, if the plan is to aspirate 50 mL of adipose tissues, then 50 mL of fluid volume or more is distributed with the adipose layer to provide the fluid carrier for extracting the grafts. A common
example of the component of tumescent solution is to add a 50 mL multidose vial of local anesthetic (eg, 0.5% to 1.0% xylocaine with or without epinephrine at 1:100,000) to 1 L sterile saline or balanced salt solution to provide sufficient tumescent fluid for lipoaspiration.

Upon completion of even distribution of tumescent fluid within the proposed donor area, repassing the infiltrating cannula throughout the donor area (termed “pre-tunneling”) multiple times is important and very helpful to attain an even and high-quality graft. This more thoroughly distributes local anesthetic fluid for patient comfort, and it also provides the needed carrier fluid to suspend the adipose tissues before harvesting with low pressure and minimal bleeding. (Note: This is a very important step that will improve comfort during harvest, make extraction more efficient, and result in markedly less volume of the unwanted infranatant fluid layer.)

In small-volume transfers, most practitioners select a 20 mL Luer syringe attached to the harvesting microcannula with a mounted locking device. A very small volume (1–2 mL) of sterile 0.9% saline is drawn into the cannula to displace air from the system before it is inserted into the harvest (donor site). This is termed “charging” the syringe device, which is necessary to eliminate all air within the cannula and syringe, thereby avoiding the cavitation produced when using mechanical pump suction devices (eg, wall suction, de-tuned lipoaspiration machines).

Once the harvesting cannula is inserted into the locally tumesced adipose layer, the syringe plunger is drawn to partial or full extension, depending on the desired vacuum pressure, and twisted to provide a lock (if using an external type) or connect to a ledge that snaps to hold the plunger in one of three positions. After application of vacuum, the physician is free to move the harvesting cannula in a forward and back series of passages. It is important that these passages are within the same plane and same pattern used during placement of the tumescent solution. At first adipose return will be somewhat slower, as the graft tissue must be in suspension for it to be easily extracted. Continuing these movements with vacuum applied will yield adipose tissues with minimal bleeding in most patients. (Note: In the event of vacuum pressure loss during the harvesting process, it is sometimes necessary to completely remove the harvesting cannula from the donor site and carefully express all air from within the cannula. When this is completed, the cannula is reinserted and the vacuum is restarted upon the pull and locking of the syringe plunger.)

Gravity will cause the yellow adipose grafts to quickly separate from the underlying (infranatant fluid), resulting in the graft’s floating on top of the small fluid volume within the syringe system. Test tube/syringe stands or decanting stands are available to facilitate this initial gravity separation (Figure 12a and b).

During the displacement of air, it is recommended that 4 × 4 sterile gauze be held over the harvesting tip openings to avoid spraying contents. Occasionally, in cases where there is a larger volume of infranatant fluid (the layer immediately below the fat tissues), simply express the liquid portion and reinsert the harvesting cannula into the donor site, lock the plunger, and gather more graft tissue.

One common cause of increased infranatant volume in the decanted syringe is inadequate distribution of local fluid, creating a pooling effect, which reduces efficiency of the adipose harvest. For this reason, extensive pre-tunneling is highly recommended before applying any vacuum to the tissues. After the desired graft volume is aspirated, the harvester cannula is removed, and the syringe is end capped and placed in a vertical position in a standard test tube rack or directly onto a decanting stand to allow gravity to separate the layers within the syringe. This usually requires decantation for approximately 2–3 minutes. After this period, practitioners expel the unwanted liquid layer on which the fat graft floats into sterile containers for disposal.

If additional graft is needed, it is possible to expel the infranatant completely and reinsert the harvester into the donor site to acquire more graft before decanting and loading the graft into the treatment syringes of choice. When the desired volume is obtained, lipoaspiration is completed, and layer separation is achieved, the transplantation syringes may be loaded via use of the anaerobic transfer (closed). The author prefers use of HD PRP as an additive to enhance the available growth factors and important signal proteins. Improved healing and maintenance of volume is the result. The typical ratio of HD PRP is 1 mL HD PRP to 9 mL of compressed autologous adipose tissue complex.

Ideally, a more thorough removal of infranatant fluids from the graft yields a more dense cellular graft. In addition, the free lipid layer (clear yellow liquid above the harvested graft) should be avoided when transplanting the autologous graft. This layer is irritating and prolongs healing of the graft tissues as it must be removed during the process by macrophages and so
Figure 12. (a) Gravity decant stand (Luer). (b) Test tube rack; gravity decant option.
Figure 13. SmartPRep II-AdiPRep Centrifuge System (Harvest-Terumo, Plymouth, Mass) (left, counterbalance [saline] weight; right, lipoaspirated adipose tissue complex before centrifugation [1000g force, 4-minute cycle]).
Figure 14. Postcentrifugation processing syringe (top layer, separator disk and free lipids [supranatant]; middle layer, compressed adipose graft [adipose tissue complex]).
Figure 15. Removal of the autologous fat graft from the centrifuged syringe (leaving the disk and free lipid for disposal).
on over time. Many practitioners recommend use of centrifugation to accomplish more ideal separation than use of gravity decanting alone (Figure 13). Centrifugation at 1000g force for 3–4 minutes is considered effective to compress the adipose tissue complex, with very clear separation of unwanted fluids (infranatant) plus isolation of undesirable free lipid layer (supranatant) (Figure 14).

In addition, some practitioners perform 1–2 rinses with sterile saline to help reduce any residual local anesthetic solution and red blood cells in specimens with slightly greater blood within the harvested grafts. It has been shown that it is not possible to completely remove the intracellular lidocaine, regardless of the number of rinsings.9

After decantation and/or centrifugation steps, the graft preparation is ready for placement into treatment syringes of the physician’s choice. It is important to use the clear anaerobic transfers (Luer-to-Luer connectors) to load the individual application syringes from the prepared, compressed graft. It is believed to be advantageous to avoid external air exposure and potential for contamination. Within the transfer options, use of Luer-to-Luer emulsification capabilities are available, which preserve the anaerobic, closed status of the grafts and permits the option of emulsification if so desired (Figure 15).

Selected treatment syringes are then mounted with the desired injection cannulas using coated, single-port cannulas. Use of blunt, coated cannulas is recommended, particularly within the facial recipient areas, to lower the risk of embolism caused by inadvertent injection of the adipose graft intravascularly. The injection cannulas are available in a variety of lengths and diameters (ranging from 0.9 mm to 1.47 mm OD), to accommodate the surgeon’s preference and the specific areas to be grafted. Some elect to inject with sharp needles ranging in size from 18 g to 25 g (Figure 16).

The typical graft recipient bed is prepared and developed by pre-tunneling to create a potential space, which is subsequently filled in small aliquots and in layers as the injection cannula is being withdrawn.

It is recommended that the donor sites be dressed in a proper fashion. Placing small, sterile gauze dressing over the actual opening created to place the tumescent fluids into the subdermal tissues will absorb any excess residual fluids displaced by adipose during the harvesting process. Further, placement of a closed-cell, medical-grade foam (TenderFoam T&N Industries, San Diego, Calif) over the entire surface above the harvested areas, with use of external compression, will eliminate or minimize post-harvest bruising of the donor area. Compression of the gauze and TenderFoam for 24–48 hours is typically effective (Figure 17a and b).

Discussion

When the mechanisms involved in liposuction technologies were recognized in the mid-late 1980s, the ability to provide small- and large-volume liposuction via the closed syringe system was proven safe and more predictably effective. Because of the even, low vacuum-pressure application offered by syringe use, enhanced abilities to provide superficial plane lipoplasty capability was proven. This included removal of significantly larger volumes in a single session and reduction of deposits within the superficial plane. This ability was thought to aid in skin redraping and improved contouring results. Besides volume implications, the syringe launched the beginning of more consistent and predictable autologous fat grafting procedures, enhancing safety, efficacy, and reproducible results within aesthetic surgical applications (Figure 18). Structural fat grafting, using the exact techniques described herein has been completed many thousands of times by many cosmetic plastic surgeons.

Science has now provided important information to help explain the homeostatic and transplant acceptance mechanisms accomplished by autologous fat. As appreciation of the biocellular nature of the adipose tissue complex increases, the importance and value of the stromal vascular fraction has garnered much attention.
It is becoming mainstream knowledge that the actual transplanted mature adipocytes are gradually lost, but they serve an important role in their own replacement from attached near terminally differentiated cells. For several years, leading practitioners sought to achieve pure adipocyte grafts without regard to the stromal vascular fraction components and the effects of the local microenvironment available with the recipient fat tissues.

The importance of signaling and growth factor secretion associated with certain paracrine effects has changed the treatment paradigm of small-volume structural grafting. It is now clear that the entire adipose tissue complex provided by lipoaspiration plays an important and integral role in achieving structural augmentation, as components participate in stimulation of the recipient site to accept the grafted cells and heal the sites. Final differentiation into metabolically active adipocytes is thought to contribute to the lipid metabolism and volume storage needed to accomplish structural augmentation using AFGs. Both the AFG and additive effects are further enhanced in the surgically damaged recipient tissues through complex signaling mechanisms of autocrine and paracrine pathways in vivo (see Figure 19a through c).

**Conclusion**

This article presents a simple and effective method of lipoaspiration to harvest adipocytes and their accompanying progenitor and stromal elements using the Tulip closed syringe system. Effective for lipoaspiration of small and large volumes, the closed syringe system and its accessories offer a full range of options to fulfill all needs for autologous fat grafting. The safety and efficacy of using the patented Tulip closed syringe system has evolved to use of a coated, disposable microcannula system specifically designed for use in structural autologous fat harvest and transfer. It currently serves as the most complete and effective gold standard for all closed syringe systems.
Figure 19.  
(a,b) Close-up view of lip augmentation 1 year after grafting with autologous fat graft (AFG) + high-density platelet-rich plasma (HD PRP) (upper lip, 3 mL total; lower lip, 2 mL total).  (c) Pre- and postoperative (20 month) views after AFG + HD PRP to lips, cheeks, and nasolabial folds (upper lips, 2.5 mL; lower lips, 2 mL; malar-submalar, 5 mL bilateral; nasolabial folds, 3 mL bilateral).  (d–g) Pre- and postoperative (2 year) views after AFG + HD PRP to the cheeks (bilateral cheeks, malar-submalar grafts, 5 mL each).
References


