Skin Rejuvenation and Volume Enhancement with the Micro Superficial Enhanced Fluid Fat Injection (M-SEFFI) for Skin Aging of the Periocular and Perioral Regions

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Abstract

Background: Adipose-derived stromal and stem cells (ADSC) in autologous fat promises regenerative advantages, and injected into the dermal and subdermal layers, enhances rejuvenation and volume. However, extremely superficial fat injection with current techniques is limited.

Objectives: Efficacy and viability evaluation of fat harvested with extremely small side port (0.3 mm) cannulae without further tissue manipulation for the correction of aging/thin skin in the periocular and perioral regions.

Methods: Micro-superficial enhanced fluid fat injection (M-SEFFI) harvests adipose tissue with a multi-perforated cannula (0.3 mm), and autologous platelet rich plasma (PRP) is added. The tissue is injected into the dermal region of the periocular and perioral zones. Efficacy and viability were evaluated by histological and cell culture analysis. Clinical assessment included retrospective evaluation according to 1 = no effect, 2 = fair effect, 3 = good effect, 4 = excellent effect.

Results: Between June 2014 and July 2015, 65 patients (7 men; mean age 49.7 years) were treated with M-SEFFI. No intraoperative complications or visible lumpiness were recorded. Analysis demonstrated mature, viable adipocytes with a strong stromal component. Following PRP addition, there was a greater proliferation noted in the M-SEFFI compared to the SEFFI (0.5 mm). Mean follow-up was 4.1 months. Clinical assessment by surgeons and patients at 1 month was 3.52 and 3.74, and 6 months 3.06 and 2.6 respectively.

Conclusions: M-SEFFI is effective and viable for lump free skin rejuvenation and volume enhancement, through the extraction of smoother ADSC rich, autologous fat tissue that does not require further tissue manipulation, to correct skin aging.

Level of Evidence: 4

Adipose tissue from autologous fat grafting is now considered the ideal filler for both volume restoration and skin rejuvenation. Fat grafting has been proven to be effective in the deep facial compartments as an ideal alternative to bioengineered fillers in volume restoration given the biocompatibility with existing tissue. Moreover, the isolation of adipose-derived stromal and stem cells (ADSC) promises regenerative, anti-inflammatory, and immunomodulatory advantages.

However, with the currently available instruments, direct fat grafting to dermic and subdermic levels for patients with thin skin often runs the risk of visibly uneven distribution.

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clotting, and occasionally vascular complications. Techniques of extensive chemical and/or mechanical manipulation of the harvested tissue have been proposed in order to achieve smoother tissue. Many have obtained smooth tissue through collagenase or extensive manipulation, which proved to be rich in stem cells but poor in adipocytes (responsible for volume restoration).\textsuperscript{7-9} Other authors have proposed other long and potentially adipocyte damaging manipulation techniques following tissue harvested by a 1 mm sideport cannula to then be injected with a very thin cannula or needle.\textsuperscript{9-12}

These techniques require special skills and awareness, such as a detailed knowledge of the vascular anatomy of the face,\textsuperscript{13} and the ability to manipulate small sized needles, syringes, and cannulae with low-pressure injections, and the slow release of the least amount of substance possible in order to avoid vascular damage.\textsuperscript{14}

The previously published SEFFI technique\textsuperscript{15} harvests adipose tissue via a rounded-tip infiltration cannula with ports of 0.8 and 0.5 mm placed along the distal cannula shaft. Previous analyses of ADSC evidenced similar growth rates and an equal tendency to differentiate into mature adipocytes for the harvested tissue. However, cells derived from a cannula with the smallest ports (0.5 mm) showed a reduced tendency to form aggregates.

We aimed to evaluate the efficacy and viability of an extension of the SEFFI technique to treat the dermic and subdermic level of the periorcular and periorbital regions, by obtaining smoother fat harvested with a multi-perforated cannula with extremely small ports (each port diameter: 0.3 mm) without post-harvest tissue manipulation.

**METHODS**

A total of 65 consecutive patients were treated with the M-SEFFI technique between June 2014 and July 2015. All patients signed an informed consent. The study was conducted in accordance with tenets of the Declaration of Helsinki and the treatment techniques were performed according to internal standardized protocols.

All procedures were performed in one of the 2 private centers, and therefore international review board approval was not required. Data were prospectively collected in a dedicated database, and included the size of the distal ports on the cannula shaft utilized for tissue harvesting, the area (s) treated with injection, and the quantity of implanted tissue for each patient. Data were retrospectively analyzed.

**Adipose Tissue Harvest and Preparation**

The preparation procedure for the SEFFI tissue harvesting technique has been previously described.\textsuperscript{15} Briefly, a solution of lidocaine (400 mg), sodium bicarbonate (5 mEq) and

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**Figure 1.** The cannulae with distal ports used for adipose harvesting for the SEFFI procedure (port diameters: 0.8 mm [left], 0.5 mm [center]), and the M-SEFFI procedure (port diameter: 0.3 mm [right]).

**Figure 2.** The tissue harvested for the SEFFI procedure with the cannula with the largest ports (port diameter 0.8; left), and the smallest ports (port diameter 0.5; center) and Micro-SEFFI (port diameter 0.3; right). The tissue harvested with the M-SEFFI technique is visibly more fluid.
epinephrine (1 mg), is prepared in a Cold Ringer's lactate solution (500 mL) and is infiltrated into the autologous collection site(s); mostly the suprapubic region (55%), hips (22%), pretocheanteric area (18%), inner thigh (3%), and inner knee (2%).

The SEFFI tissue harvesting technique specifies manual aspiration of the autologous fat with a 10-mL syringe mounted on a 20 cm long cannula with multi-perforated ports of either 0.5 mm or 0.8 mm in diameter. The M-SEFFI technique utilizes a 15 cm long multi-perforated cannula with 0.3 mm diameter ports (Figure 1). As per the standard SEFFI technique, manual micro tissue aspiration is performed 15 minutes after the infiltration of the preparation solution in the donor site.

The aspirated material (~6 cc) is then gently cleaned with cold Ringer's solution (~4 cc) and left to decanter in a vertical position for about 2 minutes. The liquid part, collected at the bottom of the syringe, is then eliminated and the whole procedure is repeated again to ensure the rinsing of the adipose tissue and the elimination of the anesthetic solution and blood, designed to facilitate fat precipitation. The syringes are capped and the remaining tissue solution (adipocytes and stromal vascular fraction [SVF]) is preserved under a sterile cloth, to reduce any potential light oxidation of adipocytes. The tissue is not centrifuged (Video 1).

Autologous blood is drawn from the patient to obtain platelet rich plasma (PRP), directly poured into four 4.5 mL citrate-containing Vacutainer tubes and centrifuged at 2000 rpm for 4 minutes. In a sterile syringe, the concentrated PRP is mixed with the harvested and washed ADSC to obtain a final concentration of 20% of the total tissue harvested. At this time, some fat tissue was sent to the laboratory for viability analysis of the ADSC components.

**The M-SEFFI Procedure**

M-SEFFI is performed as either a single procedure or in conjunction with other procedures, such as the SEFFI procedure, minimal-incisions vertical endoscopic lift (MIVEL), necklift, and/or primary or revisional blepharoplasty.

The procedure is performed under local anesthesia and monitored intravenous sedation, except for patients treated with concurrent MIVEL and necklift procedures, in which case general anesthesia is utilized.

The enhanced, ultra fluid tissue solution (Figure 2) is then injected according to a comprehensive study of the facial arterial system (Figure 3), using a linear retrograde injection technique. The tissue is injected into the dermal and subdermal level of the periocular and/or perioral regions with a 19 mm long, 27-gauge single use/disposable needle (diameter 0.361 mm and area 0.1020 mm²) on a 1 cc syringe, under the guidance of minimal digital pressure to smoothen any visible bulges (Figure 4). M-SEFFI corrects the periocular area’s radial wrinkles (Crow’s feet), upper and lower eyelid sulcus in patients with thin skin, and the naso-jugal line. In the perioral area M-SEFFI corrects the

![Figure 3](https://example.com/fig3.png)

**Figure 3.** (A) The facial arterial system. The main facial artery passes 13.5 mm (± 5.4) laterally to the labial commissure, and then passes 3.2 mm (± 4.5) laterally to the alar. It then rises up the dorsal of the nose and divides 34.8 mm from the midline and 28.8 mm from the mid-pupillary line. The artery then passes 17.4 mm from the midline and 12.9 mm from the medial ephicanthus. (B) The facial zones considered most risky for injection.
radial wrinkles (bar code), inferior lateral lines (Marionette lines), and nasolabial fold in patients with thin skin. The distal ports of the cannula used for the M-SEFFI technique are larger than the injecting needle diameter in order to eliminate any tissue manipulation (Video 2).

### Efficacy and Viability Analysis

The efficacy and viability of the M-SEFFI procedure were evaluated by previously described histological and cell culture analyses, and then compared to tissue harvested with the smallest ports of the cannula used for the original SEFFI technique (port diameter: 0.5 mm). Adipocyte viability, cell integrity and stromal components were analyzed by hematoxylin and eosin stains. The viability of the cells’ SVF was tested with Alamar Blue (AbD Serotec, Oxford, UK).

The growth rates and the tendency of differentiation of the ADSC were evaluated by culture analysis. Cells were seeded in a 24-well plate at a concentration of 25,000 cells/well and either left in Coon’s-F12 medium supplemented with 10% fetal bovine serum and 1% Glutamine for cell growth rate and differentiation analysis, or were supplemented with adipogenic induction medium (Lonza, cat. PT-3102B) and human mesenchymal stem cell (hMSC) adipogenic induction single quote (Lonza, cat. PT-4135) to induce differentiation. At 48 hours, proliferation was measured through an AlmarBlue assay, and differentiation was established following 3 weeks (according to manufacturer’s instructions).

Stromal fibroblasts were obtained after rinsing of the adipose tissue samples twice with a phosphate buffer saline solution (PBS, Sigma) and centrifuged at 1000 rpm for 1 minute to eliminate any residual Klein’s solution. The aqueous phase was discarded and the adipose tissue was digested with Collagenase I (400 U/mL; Biochrom cat. CI-22), maintained at 37°C for 60 minutes and maintained under constant agitation. Samples were then centrifuged at 1200 rpm for 5 minutes, and supernatants were discarded.

Clinical assessment was performed by means of retrospective evaluation of pre-treatment and post-treatment photographs by both the senior surgeons, and the patients. The senior surgeons assessed outcomes in terms of volume restoration and skin rejuvenation in the areas treatment with M-SEFFI. A patient satisfaction survey was administered to the patients during an in-office follow-up visit in the presence of the surgeon at 1, 3, and 6 months postoperatively, and general satisfaction for the treated areas was scored. The survey was presented whilst examining the pre- and postoperative images, together with the surgeon. Scoring was scaled according to 1 = no effect, 2 = fair effect, 3 = good effect, 4 = excellent effect. Blank copies of the survey (in Italian and English) are available as Supplementary Material at [www.aestheticsurgeryjournal.com](http://www.aestheticsurgeryjournal.com).

### RESULTS

During the study period, 65 consecutive patients underwent M-SEFFI (7 men, 58 women). The mean age was 49.7 years...
M-SEFFI was performed as single procedure in 7 cases or in conjunction the SEFFI procedure (n = 27), with SEFFI and MIVEL (n = 16), with SEFFI, MIVEL and necklift (2), with SEFFI and blepharoplasty (n = 11), or with SEFFI, blepharoplasty and necklift (n = 2).

The mean harvesting procedure time was 8.6 minutes (range, 6-23 minutes); considerably more time consuming compared to the 0.5 mm and 0.8 mm SEFFI procedures (Table 1). An average of 6.08 mL (range, 0.5-24 mL) of adipose tissue was harvested. The average implanted volume of enhanced PRP, ultra fluid adipose tissue was 7.3 mL (range, 0.6-29 mL). No intraoperative complications were recorded, including indurations, cysts, infections or any serious complications such as necrosis or fat emboli due to intravascular injection. Mean follow-up was 4.1 months (range, 1-6 months).

### Table 1. The Average Harvesting Times for the Collection of 10 cc of Tissue, According to the Three Different Cannulae used for SEFFI and M-SEFFI Techniques

<table>
<thead>
<tr>
<th>Side Port Hole Diameter (mm)</th>
<th>Average Time to Harvest 10 cc (mins, secs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>1.24</td>
</tr>
<tr>
<td>0.5</td>
<td>4.15</td>
</tr>
<tr>
<td>0.3</td>
<td>14.35</td>
</tr>
</tbody>
</table>

Histological Analysis

Tissue harvested with the smallest distal ports (diameter: 0.3 mm) was examined by histology after centrifugation and mock injection. Hematoxylin and eosin stains demonstrated mature, viable adipocytes, with intact cell walls and visible nuclei. A well-represented stromal component was noted between adipocytes, without signs of cell necrosis. The adipocyte size ranged from 0.04 mm to 0.18 mm (Figures 5 and 6).

Cell Cultures and Stem Cells Differentiation

The number of cells obtained from harvesting with the 0.3 mm diameter ports, compared to those obtained from the 0.5 mm diameter ports was inferior (Table 2). The addition of PRP instigated a greater growth rate in the cells obtained via the 0.3 mm ports (1.72 compared to 1.27) (Figure 7).

### Table 2. The Number of Cells Obtained with the M-SEFFI Technique Following Centrifugation, Prior to and After the Addition of PRP. The Data are Compared to the Number of Cells Obtained with the SEFFI Procedure with the Distal Ports (0.5 mm).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of Cells</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 M-SEFFI centrifugated</td>
<td>1.87 x 10^6</td>
<td>.014</td>
</tr>
<tr>
<td>0.3 M-SEFFI centrifugated + PRP</td>
<td>3.22 x 10^6</td>
<td>.038</td>
</tr>
<tr>
<td>0.5 SEFFI centrifugated</td>
<td>5.10 x 10^6</td>
<td></td>
</tr>
<tr>
<td>0.5 SEFFI centrifugated + PRP</td>
<td>6.52 x 10^6</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. (A) Tissue harvested with the SEFFI technique (port diameter 0.5 mm), injected into the upper eyelid. Eyelid biopsy showed mature white adipocytes (which vary only slightly in size and shape with dimensional sizes ranging from 0.04 to 0.18 mm at micrometrical ocular analysis) that infiltrate subcutaneous patchy tissue and encase skeletal orbicular fibers muscle (H&E magnification x10 times). (B) Tissue harvested with the M-SEFFI technique (0.3 mm), injected into the upper eyelid. Eyelid biopsy showed mature white adipocytes (which vary only slightly in size and shape with dimensional sizes ranging from 0.17 to 0.3 mm at micrometrical ocular analysis) that infiltrate subcutaneous organically “band like” tissue and encase skeletal orbicular fiber muscle (H&E magnification x10). The tissue injected after harvesting with the M-SEFFI technique is evidently more evenly distributed compared to the tissue injected with the SEFFI (0.5 mm) technique. Blue arrows: skeletal orbicular muscle encased by mature white adipocytes. Red arrows: subcutaneous tissue. Green arrows: mature white adipocytes infiltrated subcutaneous tissue. The injection of tissue was performed prior to upper blepharoplasty in a 48 year old female patient.
Tissue obtained by the M-SEFFI procedure is therefore more fluid with a greater rejuvenation capacity for the skin, but with a reduced volume enhancement element.

**Clinical Assessment**

Clinical assessment by the senior surgeons and the patients in terms of volume restoration and skin rejuvenation in the facial areas treated with M-SEFFI, evidenced ratings at 1 month (mean, 31 days; range, 25-37 days) of 3.52 and 3.74 (65/65), at 3 months (mean, 2.8 months; range, 2.6-3.2 months) of 3.39 and 3.17 (59/65), and at 6 months (mean, 5.8 months; range, 5.7-6.5 months) of 3.06 and 2.6 (48/65), respectively (Supplemental Table S1). Clinical photographs are shown in Figures 8-10 and Supplemental Figures S1-S4.

**DISCUSSION**

Fat tissue provides some significant advantages compared with other filler materials. It is completely biocompatible with lasting viability, it is plentiful and relatively easy to harvest. Moreover, the high rate of ADSC in fat tissue\(^{19-24}\) confers soft tissue rejuvenation properties\(^{5-6}\) and volume enhancement. Recent evidence suggests that the ADSC originates from the perivascular cells located around the blood vessels\(^{25-27}\) and the blood vessel density varies in different subcutaneous adipose tissue layers.\(^{28}\) Further, the presence of ADSC in SVF improves tissue survival.\(^{29}\) These evidences suggest that fat harvested from the more vascularized hypodermic layer may lead to improved recovery of ADSC than fat tissue harvested from deeper layers, and improve graft survival.
The manipulation of fat tissue limited in terms of volume restoration. 

It has also recently been suggested that a non-traumatic and non-damaging procedure influences not only the recovery yield of ADSC isolates but also the long term survival of the transplanted fat tissue. The manipulation of the tissue during the harvest and prior to injection into the target site can affect the viability of the tissue.

Despite the harvest of fat tissue with a micro-cannula with multiple extremely small distal ports taking considerably more time compared to that required for cannula with larger distal ports, this procedure has multiple advantages. Trivisonno et al demonstrated that harvesting fat with a micro-cannula carried a 2-fold increase in ADSC content and non-damaging procedure influences not only the recovery yield of ADSC isolates but also the long term survival of the transplanted fat tissue. The manipulation of the tissue during the harvest and prior to injection into the target site can affect the viability of the tissue.

For the patient, the mini-invasivity of the smaller cannula reduces trauma to the donor site in terms of postoperative swelling, ecchymosis, and pain. Moreover, the procedure can be performed under local anesthesia. The healing time of the microincisions is relative and the overall recovery time is reduced. For the surgeon, the multiple side-port micro-cannula permits a more precise aspiration of superficial fat from more superficial layers, harvested with more accuracy from a localized area. The risk of accidental penetration of deeper tissue and vascular damage is therefore also reduced.

A modified harvesting and injecting approach to the Coleman’s technique of utilizing a smaller gauge cannula (20-G, 23-G, or 25-G) with multi-perforated side ports (1 mm in diameter) was first introduced by pioneers such as Trepsat, Tonnard, and Alexander. Nguyen et al in 2012, experimented superficial and subdermal injections of adipose tissue harvested with a multi-perforated cannula (holes of 1 mm) in mice, and hailed the technique as a success in terms of regenerative effect of the adipose tissue and is more finely minced and thus requires less manipulation of the tissue. In turn, the risk of irregularities and visible cysts is further reduced, enabling improved regenerative effect of the adipose tissue and survival proven in other studies.

However, the use of PRP. Its addition to adipose tissue has been associated with multiple advantages, including the replacement of deep tissue and vascular damage is therefore also reduced. The SEFFI technique prescribes the harvesting of fat from more superficial layers, harvested with more accuracy from a localized area. The risk of accidental penetration of deeper tissue and vascular damage is therefore also reduced.

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Figure 9. (A, C) Pre-treatment and (B, D) 6 month post-treatment views of a 73-year-old woman treated with M-SEFFI at the nasolabial fold (2 cc per side), the fine wrinkles of the upper lid (3 cc per side), the lower lid (3 cc per side), the chin (3 cc), and the marionette lines (2.5 cc per side).
compared with a traditional (larger) harvesting cannula. Trivisonno et al demonstrated that harvesting fat with a larger distal ports, this procedure has multiple advantages. Aably more time compared to that required for cannula with multiple extremely small distal ports taking consider-
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The SEFFI technique prescribes the harvesting of fat
tissue with micro-cannula with multiple, distal ports (0.8 and 0.5) and was presented by the current authors in
2015. The harvested superficial fluid fat was proven to be
rich in stem cells and viable adipocytes. The technique in-
cludes the enhancement and improved fluidity of the tissue
by the addition of autologous PRP, proven in this study to
have a positive impact on cell growth rates and differentia-
tion. Given the enhanced skin rejuvenation and fat-graft
survival proven in other studies, M-SEFFI advocates
the use of PRP. Its addition to adipose tissue has been asso-
ciated with multiple advantages, including the replacement
of post-harvest processing, and the increased proliferation
of cellular growth. The surgeon can then harvest less
adipose tissue, thereby further reducing patient trauma.
The fluid solution is then injected into the superficial sub-
dermis with syringe needles.

The M-SEFFI technique proposes an extension of the
original SEFFI technique, harvesting tissue with smaller
ports (0.3 mm) and following the addition of PRP, the
tissue is then injected using a 27-G needle for even more
superficial filling of fine lines, wrinkles and sulcus in pa-

tients with very thin skin in the perioral and perioral area.
Manual fat emulsification, as proposed by Tonnard et al
and Stuzin et al, harvests a nanofat solution rich in SVF and
ADSCs, but without any viable adipocytes; rendering the
tissue limited in terms of volume restoration. The har-

vested fat, confirmed in this study contains viable adip-
cytes, according to histological and cell culture analyses,
and is more finely minced and thus requires less manipula-
tion in order to obtain a fluid tissue. In turn, the risk of ir-
regularities and visible cysts is further reduced, enabling
more superficial transplantation whilst profiting from the
improved regenerative effect of the adipose tissue and
volume enhancement. The use of the small sized needles,
syringes, and cannulae with low-pressure injections in the
most superficial layers and the release of the least amount
of substance possible also reduces the risk of vascular
damage.

The M-SEFFI technique compliments the SEFFI tech-
nique; SEFFI performed with cannula with distal ports of
0.8 mm is indicated for the larger volume defects in the

Figure 10. (A, C) Pre-treatment and (B, D) 6 month post-treatment views of a 49-year-old woman treated with the M-SEFFI tech-
nique at the nasolabial fold (1.5 cc per side), the upper lip fine wrinkles (2 cc per side), and the marionette lines (1.5 cc per side),
and SEFFI (port diameter: 0.5 mm) for lip enhancement (2 cc total); and SEFFI (port diameter: 0.8 mm) for malar and zygomatic
enhancement (3.5 cc per side).
cheek, temple, forehead, chin and jaw line, and SEFFI performed with cannula with distal ports of 0.5 mm is indicated for brow and lip volume restoration. We advocate the use of M-SEFFI for the upper sulcus and infra orbital hollows and the fine wrinkles in the periocular area, such as the smile lines that form in the lower eyelid, and the perioral fine lines of the lips.

Clinical evaluation of the M-SEFFI procedures were deemed by both the surgeons and the patients to have a “good effect” at 3 months and above “fair effect” at 6 months, without any registration of visible lumps or cysts or any complications. The M-SEFFI technique may also be extendable to reconstructive surgery (superficial scars, radiodermatitis, and scleroderma), other cosmetic applications (such as acne scars and rhinoplasty sequelae) and in wound healing (such as chronic diabetic ulcers and burn lesions).

However, this study is limited by the lack of a control group, which could have assisted in demonstrating any eventual superiority of the M-SEFFI compared with other treatments for the fine lines of the periocular, perioral and the superior and inferior orbital sulcus, above the muscle. M-SEFFI has only been tested with the addition of PRP, and there are no data available to suggest whether the use of the adipose tissue obtained from the micro-cannula without the addition of PRP is viable. Further, the use of a very simple outcome assessment, non-anonymous model, administered during the in-office follow-up visits in the presence of the surgeon were of minimal validity and were not accompanied by any statistical analysis. The clinical evaluation of the outcome is therefore severely limited. More valid outcome assessment models, such as FACE-Q would have improved the quality of the clinical assessment for this study.

CONCLUSIONS

The M-SEFFI technique represents a refinement of the current fat harvesting procedures, through the harvesting of smoother autologous fat tissue by means of a micro-cannula with extremely small ports of 0.3 mm diameter, mixed with PRP, for the treatment of the dermis and subdermis regions of the perioral and periocular regions, without requiring further tissue manipulation. The harvested tissue is viable, as proven at histological and cell culture analysis, has a reduced risk for lumpsiness after superficial injection, promises volume enhancement, and is appreciated by both surgeons and patients. The filler capacity of the micro fat with viable adipocytes, along with the improved fluidity and contribution to the vitality of the PRP are key to the success of M-SEFFI.

Supplementary Material

This article contains supplementary material located online at www.aestheticsurgeryjournal.com.

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