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ORIGINAL CONTRIBUTION

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A multi-center, open-label, pilot study of allograft adipose matrix for the correction of atrophic temples

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Abstract

Background: Autologous fat grafting (AFG) and synthetic fillers are currently used in esthetic and reconstructive surgery. Challenges in AFG include inconsistent graft retention, donor site morbidities, insufficient harvest, and excessive harvesting times. An allograft adipose matrix (AAM) has been developed as an off-the-shelf alternative to AFG and synthetic fillers.

Aims: To evaluate the clinical safety and retention of an AAM over 24 weeks after treatment of bilateral atrophic temples.

Patients/Methods: Ten subjects (nine females, one male, aged 47-69 years) with temple atrophy were enrolled in the IRB-approved study. AAM (Renuva®, MTF Biologics, Edison, NJ) was injected (<3 mL) bilaterally into the atrophic temples of each subject. Volume retention, global improvement, and safety were evaluated at 1, 4, 8, 12, 16, 20, and 24 weeks. Biopsy specimens were obtained for adipogenic and angiogenic histological evaluation.

Results: The mean temple volume improved over the baseline and was retained throughout the study period. Fullness (measure of volume) increased immediately from 0 pretreatment to 2.8 post-treatment (scale 0-4 = none-maximum). Fullness varied from 0.8 to 2.2 from weeks 1 through 12 and was 2.7-3.0 from weeks 16-24, around 75% increase from baseline. Furthermore, skin tone, smoothness, texture, and overall appearance also improved with 71% of subjects being satisfied to very satisfied with the results. Adverse events were minimal and histology revealed native tissue incorporation and remodeling.

Conclusion: AAM is safe and well tolerated, provides at least 6-month volume retention, improves skin quality, and supports adipose tissue remodeling after treatment into temples.

KEYWORDS

adipogenesis, allograft adipose matrix, angiogenesis, tissue graft, volume correction

1 | INTRODUCTION

Autologous fat grafting (AFG) and dermal fillers are currently used in esthetic and reconstructive surgery. Challenges to AFG include inconsistent graft retention, donor site morbidities, insufficient harvest, and excessive harvesting times. Synthetic fillers, such as cross-linked hyaluronic acid, offer only temporary benefit, although recent evidence suggests that cross-linked hyaluronic acid stimulates the production of dermal collagen and elastin.[1-3] Regarding AFG, certain applications (scars and facial contouring) may be revised with small-volume treatments[4-7] while others (breasts and buttocks) may be augmented with much larger volumes.[8-11] AFG longevity and patient outcome depend

on methods of harvesting, refinement, and placement. Properly prepared and applied autologous fat is biocompatible, versatile, stable, long-lasting, and appears natural.[9-12] In addition to body contouring, AFG improves the appearance of wrinkling and radiation damage and, to a limited extent, pore size and pigmentation. The survival and regulation of grafted adipose require a nutrient-rich environment that provides esthetic benefits which depend on underlying biokinetics.[9,10]

Esthetic contouring with small volumes of autologous fat has shown efficacy in perceptual rejuvenation of the face.[12] The drawbacks of AFG have stimulated researchers to develop an adipose-derived matrix processed from deceased human donors and suitable for allograft transplantation. Such an allograft adipose matrix (AAM) would be an off-the-shelf alternative to either augment or replace AFG and to act physically and physiologically like autologous fat without requiring a harvest site and the time and morbidity associated with harvesting.

An AAM has been developed via the processing of recovered adipose tissue as a Human Cell and Tissue Products (HCT/P) allograft. This AAM meets ISO 10 993 biocompatibility testing panel and USP <71> Sterility Testing requirements. In its final form, the AAM (Renuva®, MTF Biologics, Edison, NJ) may be stored at ambient temperature and is distributed as an off-the-shelf allograft extracellular matrix.

Preclinical studies have shown that the AAM retains, through processing, critical structural proteins as well as factors reported to support fat formation when injected in the host.[13,14] Beyond this, it has been suggested that an adipose-derived architecture may promote new blood vessel formation via infiltration of endothelial cells, creating a functional, three-dimensional scaffold into which adipocytes may differentiate and thrive.[15]

Given the long history of AFG treatments leading to a variety of clinical applications and the preclinical work demonstrating encouraging outcomes with an allograft adipose-derived matrix, we investigated the use of AAM for the correction of facial volume deficit, specifically, atrophic temples.

2 | METHODS

2.1 | Subjects

Ten subjects (nine females) aged 47-69 years (median 51.1) enrolled in the four-site, open-label study. Seven were Caucasian, 2 were Asian, and 1 was Hispanic. The Fitzpatrick Skin types were I (n = 2), II (n = 3), III (n = 4), and IV (n = 1).

Baseline atrophy was graded at 3 (n = 9) and 4 (n = 1) using the scale below.

Grade	Atrophy
0	None
1	Minimal
2	Mild
3	Moderate
4	Severe

Exclusion criteria were:

- uncontrolled systemic disease, pregnancy
- history of diabetes, HIV, keloids, hypertrophic scars; use of steroids, immunosuppressive medications, botulinum toxin, poly-L-lactic acid filler, or other dermal filler in the temple
- hypersensitivity to human collagen, history of cosmetic plastic surgery, tissue grafting, tissue augmentation with silicone, fat in the temple area
- cosmetic laser, chemical peel, intense pulsed light, or other ablative or nonablative treatment during the previous 6 months
- use of aspirin or NSAID within the previous week
- history of bleeding or connective tissue disorders and associated medications
- excessive facial exposure to sunlight or artificial UV light
- history of skin condition that might interfere with interpretation of study results
- current or recent participation in an investigational study

All subjects provided signed informed consent to participate in the IRB-approved study.

2.2 | Injection

AAM was prepared and administered to the temples in four steps: (a) application of topical anesthetic/ice or injection of lidocaine to the subject's temple treatment area, (b) preparation of syringes, (c) rehydration of the AAM tissue, and (d) injection of the AAM tissue into the subcutaneous plane of the temple. The time between anesthesia or ice application and injection of AAM was recorded. The unopened AAM product (1.5 mL) includes two sterile packages, one with a 3-mL plastic syringe preloaded with AAM tissue and the other containing an empty 3-mL plastic syringe and a sterile Luer-Lok connector. AAM was rehydrated per the provided instructions for use with 1.2 mL of saline to a final 2.7 mL (about 3 mL) of rehydrated injectable volume. The 3-mL product was ready to inject with an 18-25-gauge needle or a 19-gauge blunt cannula. The product was injected into the subcutaneous plane until the volume deficiencies were visually optimized. The exact volume injected was at the discretion of the injector but was limited to 3 mL per side. The volume injected per temple was recorded and unused product discarded. The reconstitution volume of AAM, the treatment location, and total amount of AAM injected per temple were also recorded. At the investigator's discretion, a second treatment could be given to subjects at the 8, 12, or 16-week visits.

2.3 | Objectives

The primary objective was to evaluate the temple fullness and retention of the AAM over 24 weeks after treatment into bilateral

atrophic temples of each subject, using the atrophy scoring grading scale. Secondary objectives were to evaluate the well-being and adverse events 24 weeks after AAM treatment and to evaluate biopsy-obtained histological data at the treatment site 8 to 12 weeks after treatment of AAM.

2.4 | Well-being assessments

Visits were scheduled at Day 3 (phone call), and Weeks 1, 4, 8, 12, 16, 20, and 24 for assessments of tolerability (objective and subjective), global improvement (objective, subjective), discomfort (pain), investigator-assessed efficacy, and subject satisfaction. Scales are shown below.

Subjective tolerability (burning, itching, stinging, and tingling) assessment scales (0-4)	
Objective tolerability (erythema, edema) assessment scales (0-4)	
0	None
1	Minimal
2	Mild
3	Moderate
4	Severe
Discomfort (Pain)	0 = no discomfort 5 = moderate discomfort 10 = worst discomfort ever appreciated

Investigator-assessed efficacy

Skin tone (Evenness) scale (0-4)	0 = even healthy color 4 = uneven, discolored appearance
Skin smoothness (Visual) scale (0-4)	0 = smooth appearance 4 = severe, rough appearance
Skin texture (Tactile) scale (0-4)	0 = smooth, even feeling texture 4 = rough, uneven feeling skin texture
Overall appearance of skin (0-4)	0 = healthy, youthful skin appearance 4 = poor skin appearance

Global (investigator and subject) improvement

Improvement level (+3 to −3)	Much improved	Moderately improved	Slightly improved	No change	Slightly worse	Moderately worse	Much worse
Numerical rating	+3	+2	+1	0	−1	−2	−3

Subject satisfaction

Satisfaction level (−2 to +2)	Very satisfied	Satisfied	Neither satisfied nor dissatisfied	Dissatisfied	Very dissatisfied
Numerical rating	+2	+1	0	−1	−2

Subjects were asked to rate how satisfied they were with the results of treatment to correct the fat loss of their temples according to the subject satisfaction scale. All skin attributes or parameters are mean values unless stated otherwise.

2.5 | Adverse events (AEs)

Subjects were required to report AEs at each postscreening visit. AEs were classified as mild, moderate, or severe and were determined to be not related, possibly related, probably related, or definitely related to treatment of the AAM.

2.6 | Biopsy specimens

Subjects were asked for permission to take 1 or more sub-dermal biopsy specimens at the temporal hairline or in the hair-bearing scalp at weeks 8 to 12 after treatment with the AAM. Three (3) subjects accepted. Excised samples were fixed in formalin (10%) for histopathological determination of overall tissue response, adipogenesis, and vascular growth at each implant site. Biopsy specimens were processed and analyzed by an independent laboratory for histopathology. Sections were collected at the midpoint of each implant and stained for hematoxylin and eosin (H&E). Both fluorescent and immunohistochemical (IHC) imaging were performed

for Perilipin-A (detection of functional adipocytes) and fluorescent imaging for CD31 (detection of endothelial cells). Pathologic evaluation was performed for each implant site.

2.7 | Photography

Digital photographs (full-face, right side 45°, right side 90°, left side 45°, left side 90°) were taken with a digital camera.

2.8 | Data analysis

Subject data at each time point were evaluated by simple statistics, mean ± standard deviation (SD). Paired *t*-tests were performed for treatment volumes (left vs right sides) and fullness (time zero vs 24 weeks).

3 | RESULTS

Eight patients were given a single treatment, and two patients received a second treatment. The mean ± SD for treatment volumes (mL) of the right and left sides were 1.86 ± 0.68 mL and 1.81 ± 0.67 mL, respectively. A paired *t*-test showed that the volumes (right vs left) did not differ significantly ($P = .2869$). The behavior of temple atrophy over the 24-week period of subjects who received a single AAM treatment and a second AAM treatment later in the study can be seen in Table 1.

For the single-treatment subjects (n = 8), the pretreatment atrophy (moderate) decreased from 3.0 to 1.1, a 63% reduction, at 4 weeks. The values peaked rapidly to 1.9 at week 8 (n = 7) and decreased to 0.7 or below during the remaining weeks: week 12 (n = 7); weeks 16 and 20 (n = 5); and week 24 (n = 3). The variability (SD) in values was greatest at 1 and 4 weeks, started to decrease at weeks 12 and 16, spiked at week 20, and decreased again at week 24. For subjects (n = 2), the atrophy level vacillated between 1.0 and 2.0 between 8 and 16 weeks, and then increased steadily to a peak value at 24 weeks. Variation (SD) was greatest at 1 week for both subject groups.

TABLE 1 Mean atrophy levels of single- and double-treatment subjects at each time-point of the study

	Single treatment (±SD)	Second treatment (±SD)
Week	Mean	Mean
0 (pretreatment)	3.0 (0.0)	3.0 (0.0)
0 (post-treatment)	0.3 (0.4)	0.0 (0.0)
1	1.8 (1.8)	2.0 (2.3)
4	1.1 (1.5)	1.0 (0.0)
8	1.9 (1.4)	1.0 (0.0)
12	0.7 (0.9)	2.0 (0.0)
16	0.2 (0.4)	1.0 (1.2)
20	0.6 (1.3)	1.3 (0.5)
24	0.3 (0.5)	2.3 (1.0)

Temple volume retention (or fullness) was established by taking the maximum atrophy score (4)—patient atrophy score—Time zero score (baseline). The behavior of temple volume retention (fullness) was then normalized to baseline is shown in Figure 1. For the single-treatment subjects, fullness increased immediately from 0 before treatment to 2.8 (~moderate) after treatment. For the remainder of the study, fullness varied from 1.1 to 2.3 from weeks 1 through 12 and was 2.3-2.8 (~moderate) from weeks 16-24. Of the three subjects with week 24 data, two achieved moderate fullness (3.0)) and one achieved mild fullness (2.0)). Variability was greatest at weeks 1 through 8 and decreased in the remaining weeks. For the 2-treatment subjects, fullness vacillated between 1 and 2 between 8 and 16 weeks, then decreased steadily to 0.8 at 24 weeks.

The behavior of discomfort (pain) at the treatment site during the 24-week study period is shown in Figure 2 for subjects who received a single AAM treatment and a second treatment. Overall, the levels of discomfort were low and the treatments were well tolerated. The discomfort levels decreased rapidly and approached zero at 4 weeks for the single-treatment subjects. The discomfort level of 2 for the two-treatment subjects at week 16 is due to the second treatment of 1 subject. The remaining subjects reported zero discomfort at 8 weeks and at all subsequent visits.

3.1 | Subjective tolerability

Tolerability data showed low scores and zero values 4-week post-treatment. For the single-treatment subjects, subjective tolerability (Table 2a) was generally excellent. Burning was minimal (0.9) at week 0 (treatment), barely noticeable at weeks 1 and 4, and 0 throughout the remainder of the study. Itching was less than minimal at week 0 and not observed thereafter. Stinging was initially slight up until week 4 and 0 for the remaining weeks. Tingling was less than minimal at week 0 and thereafter. Results were similar for subjects who received a second treatment (Table 2b).

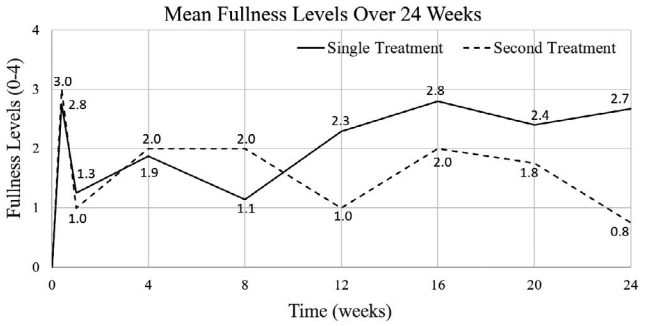

FIGURE 1 Temple fullness normalized to baseline of subjects who received a single treatment (n = 3-8) of AAM vs. subjects who received a second treatment (n = 1-2) during the study period

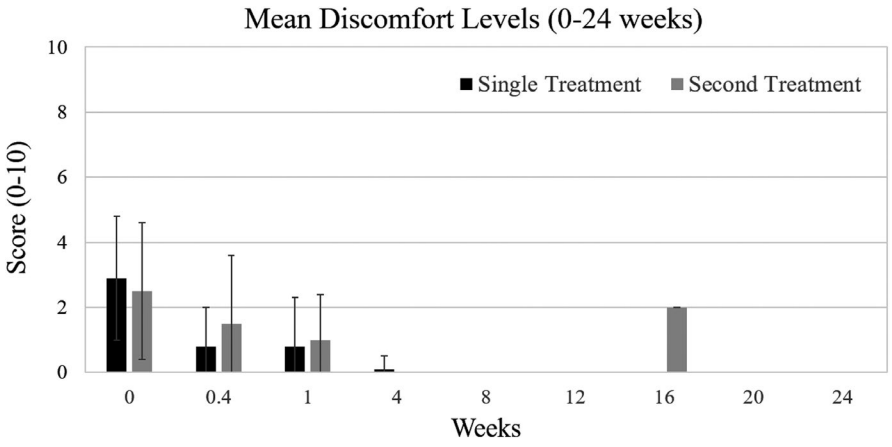

FIGURE 2 Mean discomfort levels at each time point with and without a second treatment at 16 weeks

3.2 | Objective tolerability

For single-treatment subjects, (Table 2a), erythema was less than minimal immediately after treatment, barely perceptible at week 1, and absent at each subsequent time point. Edema was below minimal immediately after treatment, less at 1 week, and resolved completely thereafter. For subjects who received a second treatment, erythema and edema were more pronounced at week 0 and 1 and resolved for the remaining weeks (Table 2b).

As stated earlier, tolerability responses included burning, itching, stinging, tingling, erythema, and edema while efficacy responses were skin tone, skin smoothness, skin texture, and skin overall appearance. As stated earlier, tolerability at the treatment sites was evaluated according to a 0 to 4 scale (0 = none, 1 = minor, 2 = mild, 3 = moderate, and 4 = severe). Percentages of responses in these categories were 0% (none), 62.6% (minor), 28.6% (mild), 3.3% (moderate), and 5.5% (severe). For tolerability responses, those greater than mild comprised 3.3% + 5.5% = 8.8%.

3.3 | Investigator-assessed efficacy

Efficacy parameters are shown in Table 3. For single-treatment subjects, skin tone ranged from 0.1 to 0.6 for the first 8 weeks and healthy (0) for the remaining weeks. Smoothness and skin texture were rated 1.1 or lower for the first 12 weeks and smooth (0) for the remainder of the study. Ranging from 0.3 to 0.9, overall appearance approached a healthy rating for the first 12 weeks, 0 at 16 and 20 weeks, and 0.2 at 24 weeks. For subjects who received a second treatment of AAM, a healthy rating was achieved much earlier, at week 1, and persisted for the remainder of the study period for each parameter.

3.4 | Global improvement

Global improvement was evaluated by the investigator (objective) and subjects (subjective). For single-treatment subjects, both objective and subjective mean improvement were positive throughout the entire study period, with improvements ranging from +0.7 up to +2.3. During the study period, assessments of improvement (objective and subjective) were made on 58 occasions and were positive on 56 (96.5%) times.

The mean subjective and objective improvement scores are shown individually in Figure 3A and B, respectively, for both single-treatment and two-treatment subjects. Subjective improvement peaked at week 16 for both groups of subjects. At 24 weeks scores suggested a trend toward a second peak following a decrease at 20 weeks. Mean scores of two-treatment subjects were consistently higher than those of single-treatment subjects.

Objective improvement peaked at 4 weeks and remained relatively constant and high for both groups of subjects. Improvement behaviors were less variable during the study period compared to subjective behaviors. In contrast to subjective scores, objective scores for single-injection subjects were consistently higher than scores of two-treatment subjects after week 8. Clinical images of subjects are shown in Figures 4-5.

3.5 | Subject satisfaction

Five single-treatment subjects rated their satisfaction with the results of treatment to correct the fat loss of their temples. The results are shown in Figure 6. One subject (no. 4) was very satisfied, 2 subjects (nos. 1, 3) were satisfied, and 2 (nos. 2, 5) were neither satisfied nor dissatisfied. Sixty percent (60%) were satisfied or very satisfied, and no subject was dissatisfied. For subjects who received a second treatment, one was very satisfied and the other was satisfied.

3.6 | Histology

Five subjects consented to biopsy for histological evaluation. Two biopsy specimens (YK and SR) were taken 8 weeks after a single treatment, two (KV and JF) at 12 weeks, and another (TL) at 16 weeks (after a second treatment). The results are shown for YK, TL, and KV in Figures 7-9, respectively.

TABLE 2 (a) Tolerability (subjective and objective) throughout the study period for subjects who received a single treatment of AAM. (b) Tolerability (subjective and objective) throughout the study period for subjects who received a second treatment

Week (n = no of subjects)	Subjective mean (±SD)				Objective mean (±SD)	
	Burning	Itching	Stinging	Tingling	Erythema	Edema
(a)						
0 (8)	0.9 (1.0)	0.3 (0.6)	0.3 (0.9)	0.1 (0.3)	0.8 (1.0)	0.8 (0.8)
3 days (8)	0.0 (0)	0.0 (0.0)	0.1 (0.5)	0.0 (0.0)	—	—
1 (8)	0.1 (0.3)	0.0 (0.0)	0.2 (0.5)	0.0 (0.0)	0.1 (0.3)	0.5 (1.4)
4 (8)	0.1 (0.3)	0.0 (0.0)	0.1 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
8 (7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
12 (7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
16 (5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
20 (5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
24 (3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
(b)						
0 (2)	0.3 (0.5)	0.0 (0.0)	0.3 (0.5)	0.0 (0.0)	2.0 (0.0)	1.0 (0.0)
3 days (2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)		
1 (2)	0.0 (0.0)	0.5 (0.6)	0.0 (0.0)	0.0 (0.0)	2.0 (0.0)	2.5 (0.6)
4 (2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
8 (1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
12 (1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
16 (2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
20 (2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
24 (2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

In subject (YK), Figure 7, H&E images (4x and 10x magnification) indicate the presence of adipocytes within the AAM, 8 weeks after treatment. Perilipin-A fluorescent staining reveals the presence of Perilipin-A (red stain), a lipid protein surrounding viable adipocytes,[16] and CD31 staining indicates the presence of new blood vessels (CD31-positive cells red staining) and nuclei (DAPI-blue staining). DAPI (4′, 6-diamidino-2-phenylindole) is a DNA-specific probe that forms a fluorescent complex with adenine-thymidine sequences of DNA[17] in the nucleus.

The presence of Perilipin-A positive cells, the red-stained new blood vessels (CD31-positive cells), and the blue DAPI-stained nuclei indicate that 8 weeks after AAM treatment, the implant has begun to facilitate host cellular infiltration and support both adipocyte and endothelial cellular repopulation. Temple fullness of this subject was three (moderate atrophy) at both pretreatment (baseline) and 8 weeks after treatment, suggesting that although cellular remodeling had begun, 8 weeks after treatment may be too early to observe an actual increase in temple fullness compared to baseline. However, at 8 weeks, the Subjective Global Improvement score was 2.0 (moderately improved) and Objective Global Improvement was 1.0 (slightly improved), indicating that improvement compared to baseline was noticeable. Skin tone improved from 1 at pretreatment to 0 at 8 weeks; skin smoothness and skin texture were 1 at both pretreatment and 8 weeks, the overall appearance was 1 at pretreatment and 0 at 8 weeks, and subjective global improvement was 1.0 (slightly improved) at 24 weeks.

In the second subject (KV), Figure 8, H&E staining highlights the presences of adipocytes 12 weeks after treatment and positive Perilipin-A staining verifies the presence of functional adipocytes (IHC-brown Perilipin-A and fluorescent red staining). In addition, CD31 verifies the presence of new blood vessels (CD31-positive cells red stain) and nuclei (blue DAPI staining).

The presence of Perilipin-A positive cells, the red-stained new blood vessels, and the blue DAPI-stained nuclei indicate that 12 weeks after AAM treatment, the implant is facilitating host cellular infiltration, adipogenesis, and angiogenesis. Temple fullness of this subject was 3 (moderate atrophy) at pretreatment (baseline) and 0 (no atrophy) at 12 weeks after treatment, indicating that cellular remodeling was taking place and an actual increase in temple fullness compared to baseline is observed. At 12 weeks, Subjective Global Improvement was 1.0 (slightly improved) and Objective Global Improvement was 3.0 (much improved), indicating that improvement compared to baseline was apparent. Pretreatment data for skin tone, skin smoothness, skin texture, and overall appearance were not available. Immediate post-treatment scores were 0 for these four attributes and all remained unchanged at 12 weeks.

In subject (TL), Figure 9, H&E images (4x, 10x, 20x magnification) also indicate the presence of adipocytes and the probable residual AAM near adipocytes 16 weeks after treatment. Two methods of Perilipin-A staining were examined (fluorescent red and IHC-brown). The presence of Perilipin-A and the apparent presence of residual AAM near adipocytes suggest that AAM was in position to initiate host cell infiltration and facilitate adipocyte and endothelial cellular

TABLE 3 Mean skin tone, skin smoothness, skin texture, and overall appearance scores at treatment site after a single and second treatment of AAM

Weeks (n = no of subjects)	Tone (±SD) (0-4, 0 = Healthy to 4 = Uneven)		Smoothness (±SD) (0-4, 0 = Smooth to 4 = Rough)		Texture (±SD) (0-4, 0 = Smooth Feeling to 4 = Rough feeling)		Overall appearance (±SD) (0-4, 0 = Healthy to 4 = Poor)	
	Single treatment	Second treatment	Single treatment	Second treatment	Single treatment	Second treatment	Single treatment	Second treatment
0 (8)	0.5 (0.5)	0.0 (0.0)	0.6 (0.5)	0.0 (0.0)	0.6 (0.5)	1.0 (0.0)	0.6 (0.5)	1.0 (0.0)
1 (8)	0.1 (0.3)	0.0 (0.0)	0.1 (0.3)	0.0 (0.0)	0.4 (0.7)	0.0 (0.0)	0.5 (0.8)	0.0 (0.0)
4 (8)	0.6 (0.9)	0.0 (0.0)	0.8 (0.9)	0.0 (0.0)	0.9 (1.0)	0.0 (0.0)	0.8 (0.9)	0.0 (0.0)
8 (7)	0.6 (1.5)	0.0 (0.0)	1.1 (1.4)	0.0 (0.0)	1.1 (1.4)	0.0 (0.0)	0.9 (1.4)	0.0 (0.0)
12 (7)	0.0 (0.0)	0.0 (0.0)	0.3 (0.5)	0.0 (0.0)	0.4 (0.5)	0.0 (0.0)	0.3 (0.7)	0.0 (0.0)
16 (5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
20 (5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
24 (3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.4)	0.0 (0.0)

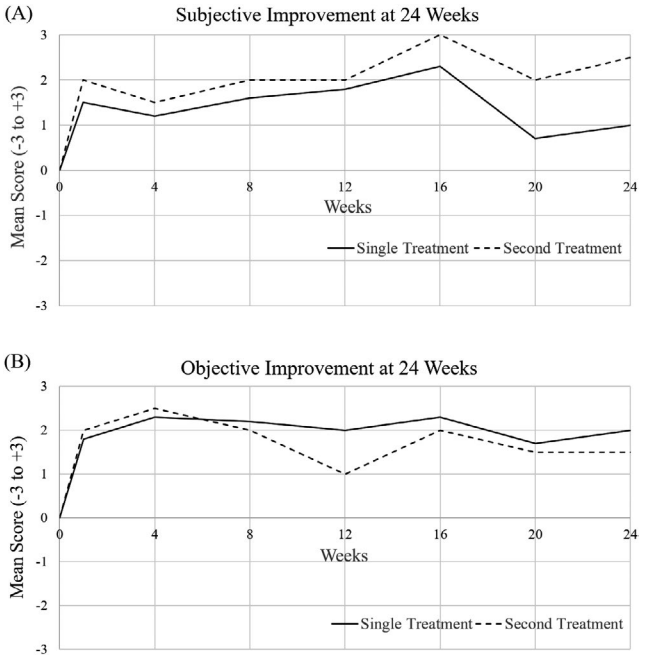

FIGURE 3 A, Mean subjective improvement of subjects who received a single treatment of AAM and a second treatment (~16 weeks). Improvement behaviors were parallel throughout the study period, although improvements were consistently higher in subjects who received a second treatment (~16 weeks). Scale: +3 = much improved, −3 to much worse. B, Mean objective improvement of subjects who received a single treatment of AAM and a second treatment (~16 weeks). Improvement behaviors were parallel throughout the study period, although after week 8, improvement scores were generally higher in subjects who received a single treatment. Scale: +3 = much improved, −3 to much worse

repopulation. At 24 weeks, temple fullness was 1 (right, minimal atrophy) and 2 (left, mild atrophy), global improvement was 3.0 (subjective, much improved) and 2.0 (objective, moderately improved), and efficacy was 0 for skin tone (even), smoothness (smooth), texture (smooth feeling), and overall appearance (healthy).

3.7 | Adverse events

Serious adverse events were not observed, and the small number of reported adverse events was limited to bilateral temporal pain (n = 2), temple bruising on one side (n = 2), swelling (n = 1), and nausea (n = 1). All events were reported during the Day 3 telephone call and resolved by the Week 1 office visit. During the Week 1 visit, periorbital swelling was observed in a single subject and resolved using massage without complication.

4 | DISCUSSION

The results show that the AAM is safe, well tolerated, and efficacious in the temple when administered in the amounts (~2 mL) of the

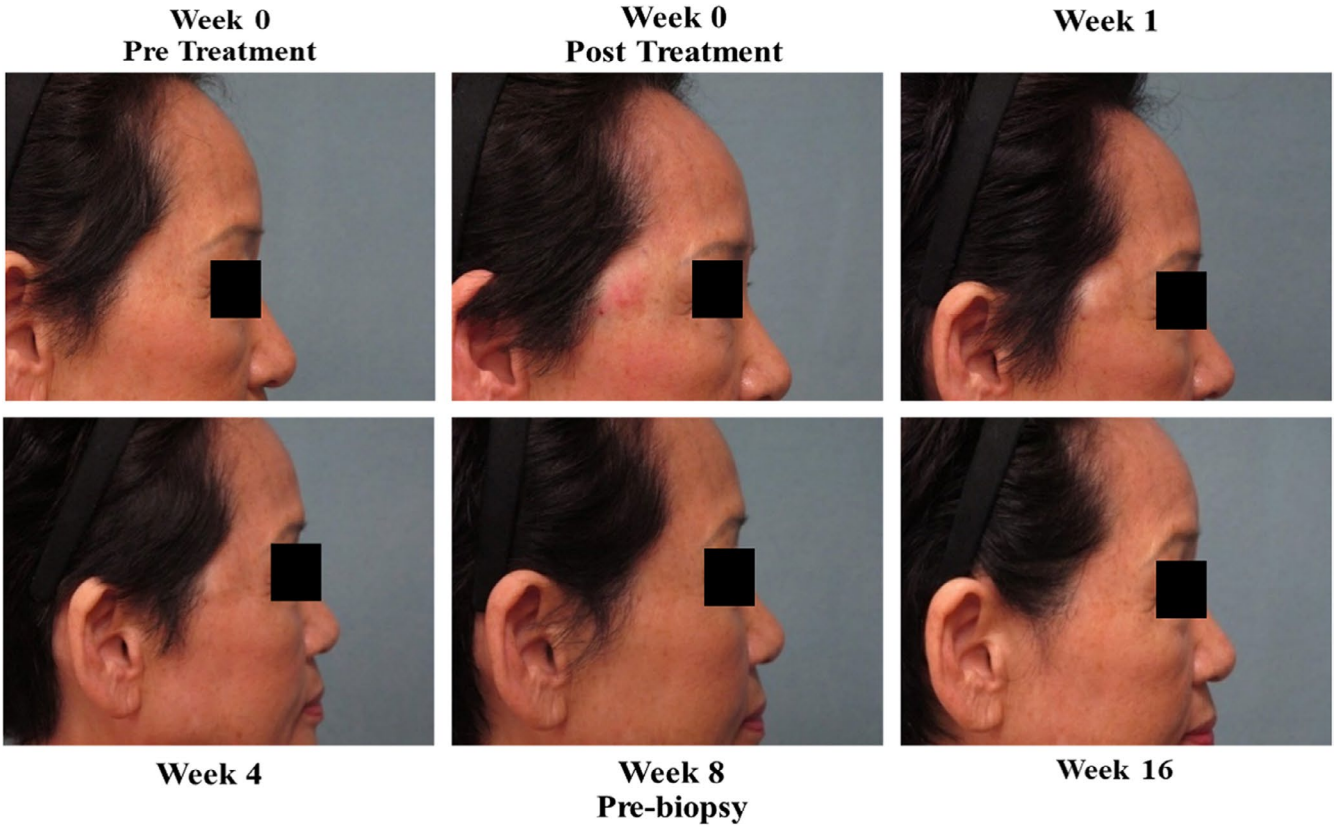

FIGURE 4 The right temple of a 69-year-old Asian female of skin type II. The subject's pretreatment atrophy score was three (moderate atrophy) and her week 24 score as 0 (no atrophy). The bulge due to allograft adipose matrix is close to the hairline at week 0 (immediately after treatment) and gradually reduces in size until after week 8, where it is less conspicuous as the contours of the temple improve. Subject satisfaction with the results was +1 (satisfied) at week 20 and +2 (very satisfied) at week 24 for this subject

FIGURE 5 A 47-year-old Caucasian female of skin type III. The front view shows the right and left temples before treatment of allograft adipose matrix and at weeks 8, 16, and 24, showing gradual improvement in fullness of both right and left temples. Pretreatment atrophy was 3 (moderate) and week 24 atrophy was 1 (minimal). Global improvement (subjective and objective) was greatest between weeks 8 and 16 but noticeable at weeks 20 and 24. Subject satisfaction with the results was +2 (very satisfied) at weeks 20 and 24

present study as evidenced by measurements of volume retention up to 6 months and adipogenesis at the site of treatment.

4.1 | Safety

Serious adverse events were not observed, and the small number of reported adverse events was limited and minor and resolved within a week.

In the present study, the mean discomfort level at the treatment site was less than moderate (2.9 in which 0 = no discomfort, 5 = moderate discomfort, 10 = worst discomfort) immediately after treatment (Figure 2). Discomfort was minimized with use of topical anesthetic, ice, or treatment of lidocaine. The mean level decreased rapidly to less than 1.5 and resolved completely well before the end of the study. Other adverse events were temporary and limited to bilateral temporal pain, temple bruising, swelling, and nausea. All were resolved by the week 1 office visit. Overall tolerability was generally excellent, with discomfort being minimal or barely noticeable 1-4 weeks post-treatment and nonexisting after that.

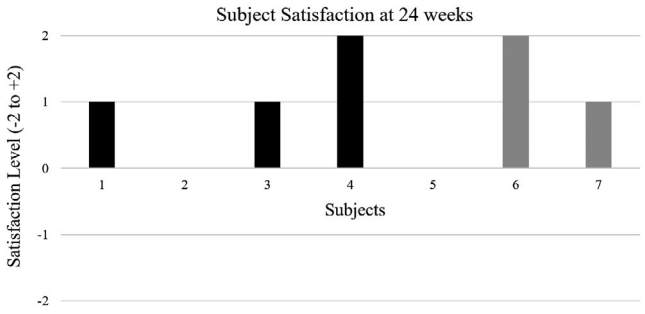

FIGURE 6 Satisfaction of each of five subjects (one treatment, no. 1-5) and two subjects (two treatments, nos. 6,7) at treatment site 24 weeks after treatment of AAM

In contrast, adverse events are known to occur with all dermal fillers.[18] Hyaluronic acid has been associated with necrosis,[19-22] migrating granuloma,[23] hypersensitivity,[24] inflammation, and hyperpigmentation.[25] Calcium hydroxylapatite has been linked to necrosis,[26,27] filler displacement,[28] and pain.[29] Poly-L-lactic acid has been accompanied by granuloma[30] and pain.[31] Polyacrylaminde gel has been associated with granuloma[32] and bacterial infection[33] and polymethylmethacrylate has been linked to granulomas.[18] None of these adverse events were observed in the present study.

Safety concerns regarding treatment of the temple area have been raised due to proximity to the ophthalmic artery. The concern arises from crossover arborization of the supraorbital and supratrochlear arteries that have retrograde flow to the retinal branch of the ophthalmic artery. Obstruction of ophthalmic artery may lead to blindness.[34] Beleznay et al,[35] in their comprehensive review, stressed that blindness could result from injecting virtually any facial area and that cases have involved autologous fat, hyaluronic acid collagen, paraffin, PMMA, silicone oil, poly-L-lactic acid, calcium hydroxyapatite, and polyacrylamide hydrogel. More recently, Thanasarnaksorn et al[36] reported vision loss in six patients due to treatment of hyaluronic acid into the nose, forehead, and temple.

Visual events were not observed in the present study. In the authors' opinion, the key to avoiding intravascular treatment is the use of meticulous superficial technique in an antegrade and retrograde manner. Treatment is gradual, low-pressure, and with constant to-and-fro motion. The authors also strongly recommend that physicians do not inject synthetic fillers into a previously treated temple area due to the high risk that abnormal vasculature anatomy may be present in these patients.

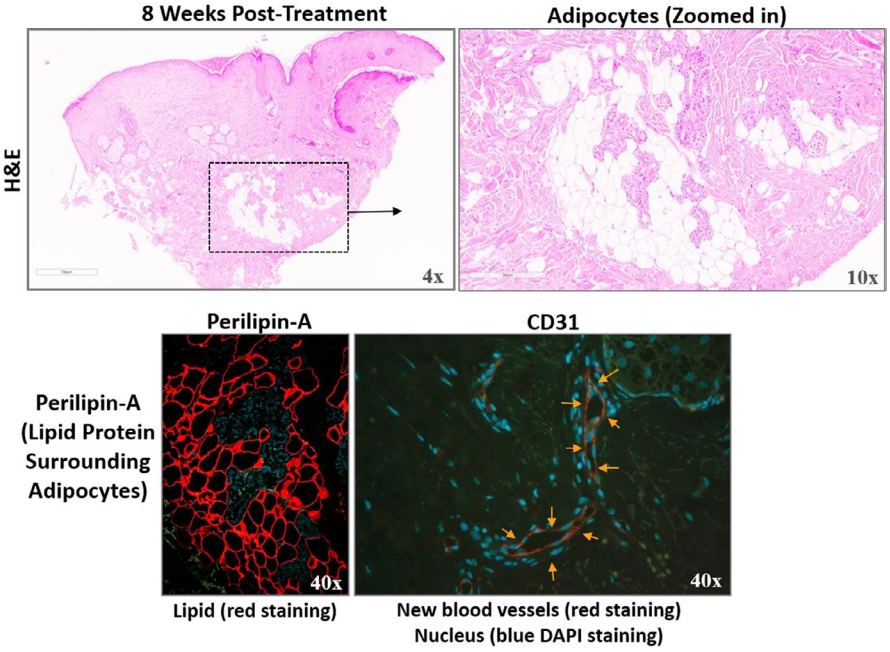

FIGURE 7 Biopsy of subject (YK) taken and analyzed at 8-week post-treatment of AAM. Presence of adipocytes (Perilipin-A positive red staining surrounding adipocytes) and endothelial cells (presence of CD31-positive red staining) was observed

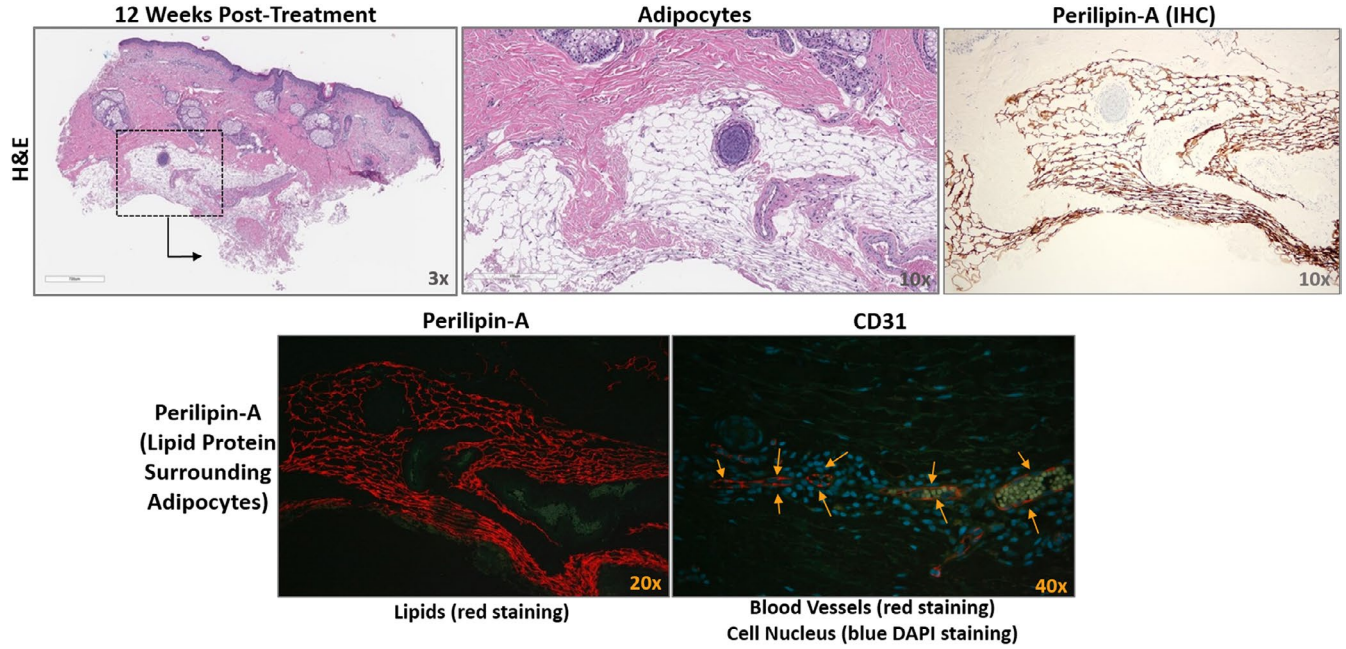

FIGURE 8 Biopsy of subject (KV) taken and analyzed at 12-week post-treatment of AAM. Adipocytes, positive staining of Perilipin-A (lipid protein that surrounds adipocytes) and endothelial cells (presence of CD31-positive red staining) was observed

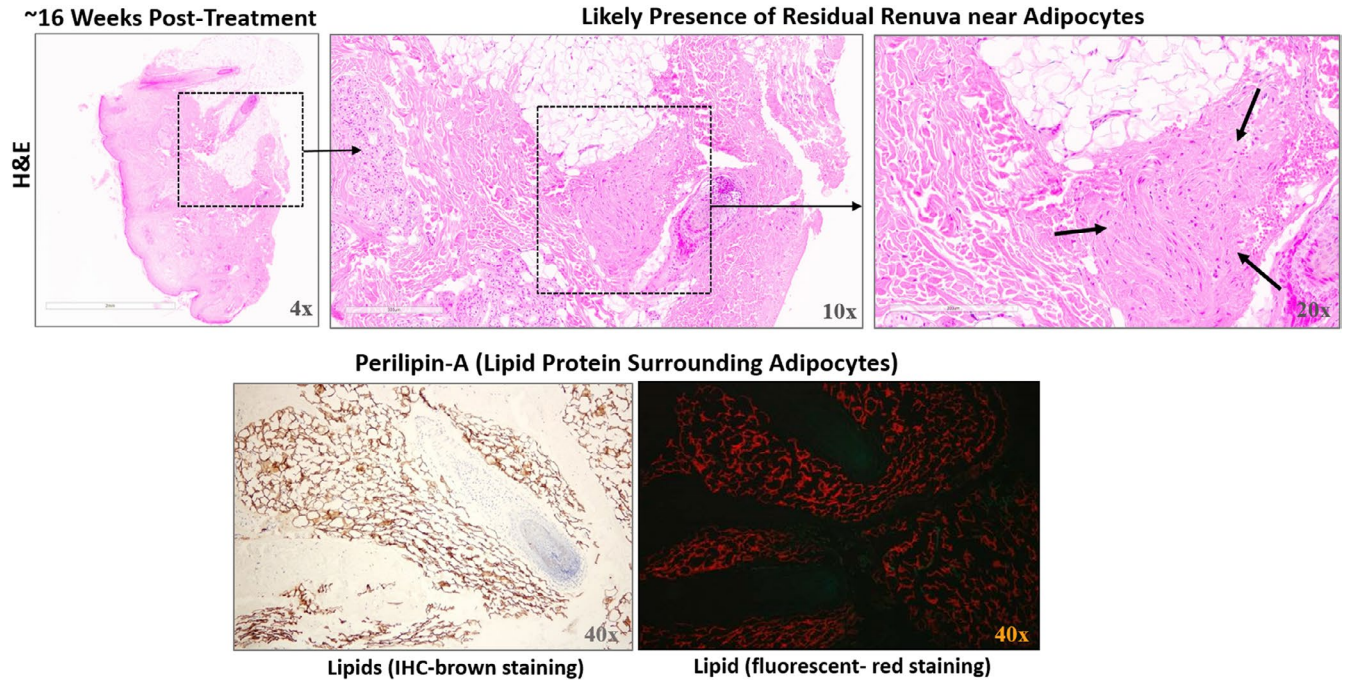

FIGURE 9 Biopsy of subject (TL) taken and analyzed at 16-week post-treatment of AAM. A second treatment was administered at week 8. Adipocytes were observed through positive Perilipin-A staining (lipid protein surrounding adipocytes). Presence of residual AAM (blue arrows) can be seen near the adipocytes

4.2 | Tolerability

As stated in the results, tolerability responses greater than mild comprised of 3.3% + 5.5% = 8.8%. This may be verified by comparing the number of greater than mild incidents to the total number of opportunities for an incident to occur. The number of greater than mild tolerability incidents was 16, and the total number of opportunities is 1800 (10 subjects × two temples × nine visits × 10 responses (six tolerability and four efficacy)). The percentage of greater than mild incidents = (16/1800) × 100 = 8.8%. In contrast, the incidence of greater than mild adverse events for Juvederm has been reported at 25%,[37] 82%,[38] and 29.9%[39] and for Restylane at 96.3%[38] and 43.1%.[39]

4.3 | Longevity

Temple fullness (Figure 1) persisted throughout the 24-week study period for most patients who completed the study. As shown in Figure 1, a second treatment during the study may not prolong fullness in all patients. This may be related to the variability between subjects, and how each individual may respond to the treatment, even if the treatment may be proven to be efficacious for a large percentage of subjects. Improvement, however, persisted for at least 24 weeks in some patients. Subjective improvement throughout the study was greater in patients who received a second treatment (Figure 3A) while the opposite was true with objective improvement (Figure 3B). That said, longevity of the AAM appears to be at least 6 months.

As stated earlier, preclinical studies show that injectable AAM retains key structural proteins as well growth factors which have been shown to support adipogenesis and angiogenesis and necessary for host fat formation.[13] That said, the behavior of atrophy (Table 1) and fullness (Figure 1) throughout the 24-week period may be understood by referring to the prebiopsy photographs at weeks 0 through 16 (Figure 4), weeks 0 through 24 (Figure 5), and the postbiopsy micrographs of subjects at weeks 4, 12, and 16 (Figures 7-9, respectively).

In Figure 5, the subject's pretreatment atrophy was moderate (minimal fullness). Immediately after treatment the bulge due to AAM became visible and gradually became smaller until after week 8. Fullness (Figure 1) of the subject pool showed a corresponding peak at week 0 immediately after treatment. This was followed by a gradual reduction in fullness until week 8 as the AAM began to facilitate host cellular infiltration and support blood vessel formation and autologous fat formation. These results from this study demonstrate that AAM contains factors that support autologous angiogenesis and adipogenesis. After week 8, fullness began an upward trend as cellular remodeling continued (Figure 7). The trend continued through week 12 (Figure 8) as host cellular infiltration, autologous adipogenesis and angiogenesis continued. At week 16 (Figure 9), lipid continued to accumulate around the adipocytes. At weeks 16 and 20, fullness reached its immediate post-treatment value (3.0) and declined slightly to 2.7 (90% of its peak value) at 24 weeks.

The duration of effects for hyaluronic acid fillers has been reported at 3 to 12 months,[40] 1 year,[41]18 months,[42] and 2 years.[43] A recent study of the use of injectable hyaluronic acid gel to correct volume loss of facial temporal fossa showed that the effects of the gel persisted at least 12 months in 98% of subjects.[34] Although cross-linked hyaluronic acid also stimulates the production of dermal collagen and elastin,[1-3] there is no evidence that this synthetic filler retains critical structural proteins as well as key angiogenic and adipogenic factors that support fat formation when injected in the host. These unique properties of AAM suggest that the AAM creates a functional, three-dimensional scaffold into which adipocytes may differentiate and thrive.[15] This theory is supported by histopathological observations of the present study as well as of a prior clinical study where the same tissue matrix (AAM) was used in the dorsum of the wrist.[13]

Interestingly, the overall behavior of the AAM as exemplified by the shape of the fullness curve in Figure 1, appears to follow the same trend as when AFG is used to fill defects for volume restoration. An immediate fill and increase in volume may be observed, followed by a slight decrease, followed quickly by final volume restoration.[44-46] Although the timeframe for volume restoration and retention between AAM and AFG may be different, AAM in this study appears to be effective in a shorter amount of time. The data may suggest similarities in the underlying mechanism of action for the two grafts.

4.4 | Efficacy

Both objective and subjective improvement were positive throughout the entire study period for 91.7% of assessments. All subjects achieved objective and/or subjective improvement up to their final week 12 (n = 2), week 20 (n = 1), week 12 (n = 2), week 20 (n = 1), and week 24 (n = 3). Global improvement data were not available for two subjects.

Seven subjects rated their satisfaction with the results of AAM treatment to correct fat loss of their temples. Seventy-one percent were satisfied or very satisfied, and no subject was dissatisfied. Overall, the subjects in this study experienced improvements from the AAM treatment and benefited from this off-the-shelf option. The outcomes of this study are comparable to those achieved in previous studies when AFG was used, without the need for the secondary and painful procedure of harvesting the subject's fat.

Skin tone, skin smoothness, skin texture, and overall appearance were favorable up to weeks 8 to 12 and were healthy for the remaining weeks (score of zero). This limited data provide early insight into the potential for AAM to positively impact skin quality and might hint to the potential of AAM to affect skin restoration. Similar observations have been made when AFG is used in facial applications.[44-46]

Limitations of the present study are the small number of subjects and the absence of complete data on some subjects.

5 | CONCLUSION

The AAM is safe and well tolerated in the temple, provides at least 6 months volume retention, and supports autologous fat formation and tissue remodeling after treatment. These encouraging results justify larger studies to further evaluate the safety and efficacy of the AAM for the correction of facial volume deficits.

DISCLOSURES

Drs. Gold, Kinney, Kaminer, and Rohrich are paid consultants and perform clinical research for MTF Biologics. Dr D'Amico is a paid consultant for MTF Biologics.

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