Effects of Monopolar Radiofrequency Treatment Over Soft-Tissue Fillers in an Animal Model: Part 1

Laura J. England, PhD,1 Mei-Heng Tan, MD,2 Peter R. Shumaker, MD,3 Barbara M. Egbert, MD,4 Kim Pittelko, HT (ASCP),1 David Orentreich, MD,2 and Karl Pope, MS1*

1Thermage, Inc., 25881 Industrial Boulevard, Hayward, California 94545
2Orentreich Medical Group, New York, New York 10021
3Naval Medical Center, San Diego, California 92134
4VA Palo Alto Health Care System, Palo Alto, California 94394

Background and Objectives: Monopolar radiofrequency (RF) treatment is used by physicians to tighten and contour the skin of their patients. In many cases, patients have received prior treatment with other aesthetic modalities such as soft-tissue augmentation or they may wish to receive these treatment modalities simultaneously. Together, soft-tissue augmentation and monopolar RF treatment have the potential to restore tissue volume and improve facial laxity. To date, no published studies have documented the effects of RF treatment directly over soft-tissue fillers.

Study Design/Materials and Methods: We examined the tissue interactions of monopolar RF heating with five commonly injected fillers in a juvenile pig model. This is the first part of a two-part study. In this study, the interaction of monopolar RF and filler substances was examined over a period of 4 months. The five soft-tissue fillers examined were cross-linked human collagen (Cosmoplast™), hyaluronic acid (Restylane®), calcium hydroxylapatite (Radiesse™), polylactic acid (Sculptra™), and liquid injectable silicone (Silikon™ 1000).

Results: There was no apparent increase in the risk of local burns and no observable effect of RF treatment on filler persistence in the tissue. With monopolar RF treatment, an increase in fibroplasia and collagen deposition surrounding Restylane, Radiesse, and Sculptra was observed. When scored in a blinded fashion, the increase in collagen deposition was statistically significant for Radiesse.

Conclusions: In this animal study, RF treatment had no observed adverse effect on filler collagen responses or persistence. Filler presence did not increase the risk of undesirable thermal effects with monopolar RF treatment. Further clinical studies are required to evaluate the effect of monopolar RF treatment over dermal fillers with respect to aesthetic outcome. Lasers Surg. Med. 00:1–10, 2005.

INTRODUCTION

The ThermaCool TC™ system (Thermage®, Inc., Hayward, CA) is a non-ablative monopolar radiofrequency (RF) technology that has 510(k) clearance for the full-face treatment of wrinkles and rhytids. During treatment, RF energy is delivered into the skin while cryogen (R134a) cools the treatment tip surface resulting in volumetric heating of the dermal and sub-dermal tissue while protecting the epidermal layer from thermal damage. Physicians use the monopolar RF technology to tighten and contour the skin of their patients.

The device uses a treatment tip that employs a capacitively coupled monopolar electrode as the active electrode. A return electrode is placed on the body, typically on the back. RF delivery through the treatment tip creates an electric field under the electrode. As the charge of the electrode changes from positive to negative at a rate of six million times per second (6 MHz), electrons and ions are either attracted or repelled from the electrode surface. Resistance to the movement of the charged ions in the skin and subcutaneous tissue causes heat. The heating depth of the electrode is dependent on the geometry of the treatment tip and can be controlled from a few hundred microns to millimeters in depth. The device currently uses 1.0- and 1.5-cm² treatment tips both having a medium treatment depth, which have been shown to heat tissue up to 8 mm in depth. These tips have been used to treat cheek, neck, brow, and moderate to severe acne vulgaris [1–5].

Studies indicate that tissue tightening occurs through a mechanism of immediate collagen contraction, supplemented by new collagen synthesis during a longer-term wound-healing process. Ultrastructural analysis of human tissues immediately after treatment revealed isolated, scattered areas of denatured collagen fibrils with increased diameter and loss of distinct borders [6]. Collagen fibrils, when
heated, will denature and contract, which is believed to lead to the observed tissue tightening. Monopolar RF treatment has also been demonstrated to induce a wound-healing response in an animal model resulting in fibroplasia, and increased collagen [7]. Increased collagen gene expression has also been demonstrated in monopolar RF-treated human skin [6].

Soft-tissue filler augmentation is a treatment modality widely used for aesthetic improvement of deep facial lines and wrinkles. Substances used fordermal filling vary with respect to durability, cost, convenience, and type of correction desired (reviewed in Refs. [8,9]). The filler substances used in this study are briefly described below.

Cosmoplast™ (Inamed Corp., Santa Barbara, CA) is highly purified collagen derived from human fibroblasts with FDA clearance fordermal implantation since 2003. Cosmoplast is cross-linked with glutaraldehyde to increase its longevity in injected tissue. Because collagen is naturally occurring in the body, human-derived purified collagen injectables are therefore highly biocompatible [10]. Injected collagen is fairly rapidly resorbed with loss of clinical effect and histological presence by 3–6 months.

Restylane® (Q-Med, Uppsala, Sweden) is a form of hyaluronic acid, a naturally occurring mucopolysaccharide present in the dermis. Restylane is biosynthetically produced by bacterial fermentation and subjected to molecular cross-linking to make it more resistant to heat and enzymatic degradation. Restylane obtainedFDA clearance for dermal implantation in 2003 and is widely used to correct facial wrinkles and folds. Restylane is somewhat longer-lasting than collagen with correction lasting approximately 3–9 months. Both collagen and hyaluronic acid implants augment tissue primarily by adding volume.

Sculptra™ (Dermik Laboratories, Berwyn, PA) is an injectable form of poly-L-lactic acid. Polylactic acid is a naturally occurring member of the alpha-hydroxy acid family and is synthesized from corn-derived material. Sculptra particles are 40–63 μm in diameter and are formulated with carboxymethylcellulose and mannitol. Polylactic acid has been widely used for many years as a component of absorbable sutures and implantable screws. Sculptra was cleared for use in the US in 2004 for treatment of facial wasting in HIV patients. Correction is achieved over several injection sessions and lasts at least 2 years.

Medical-grade liquid silicones are long polymers of dimethylsiloxanes. Silikon™ 1000 (Alcon Laboratories, Fort Worth, TX) is medical-grade liquid silicone with a 1,000 centistokes viscosity. It is FDA-approved as an ophthalmic device for certain cases of complicated retinal detachment. Although it does not have clearancefor dermal implantation, it has been used off-label for permanent soft-tissue augmentation. Implanted silicone droplets augment tissue volume and promote collagenesis over a period of months.

It is estimated that over half of patients treated by aesthetic specialists undergo multiple procedures in the same year, with filler injections ranking among the most common and fastest growing procedures [15]. Together, soft-tissue augmentation and monopolar RF treatment have the potential to restore tissue volume and improve facial laxity. No studies exist, to date, that document whether RF treatment directly over filler substances increases the risk of thermal burns or has an adverse effect on implant longevity in the tissue. This study describes the first examination of the effects of monopolar RF treatment over a variety of soft-tissue fillers in a juvenile pig animal model.

Filled tissue areas were RF treated immediately or 1 month following injection of five different fillers: cross-linked human collagen, hyaluronic acid, calcium hydroxylapatite, poly-L-lactic acid, and liquid silicone. Immediate tissue responses to RF treatment over fillers were monitored. Relatively high treatment levels were employed to enable the detection of potential adverse effects of RF treatment over fillers. Filled and/or treated areas were biopsied at various time intervals over a 4-month period and examined for filler presence and for the histological response of the filled tissue area to RF treatment. The second part of this study examines effects of more physiological RF treatment levels up to 1 month post-treatment [16].

MATERIALS AND METHODS

Two female juvenile Yorkshire pigs were treated in an ethics-approved animal study. The animals were maintained under anesthesia during tattooing, injection, and/or RF treatment. One week prior to study commencement, the animals were permanently marked with India ink in the corners of a 1.5 cm² grid over the entire abdomen, excluding over the ribs and sternum. This marking lasted throughout the study period and allowed identification of individual treatment square locations. The dermal fillers Cosmoplast, Silikon 1000, Restylane, Radiesse, and Sculptra were injected in their commercially available formulations into marked squares on the abdominal skin of the pigs. Cosmoplast was preformulated with 0.3% lidocaine. The other filler substances did not contain anesthetic agents. Sculptra lyophilisate was resuspended in 3 ml sterile water. Each
1.5 cm² treatment square was injected with 0.2 cc of Silikon 1000 using a microdroplet serial injection technique or 0.25 cc of Cosmoplast, Restylane, Radiesse, or Sculptra using a linear threading technique [17]. With Cosmoplast, Sculptra, Radiesse, and Restylane, this resulted in the addition of approximately 2 mm depth of introduced filler material. Cosmoplast and Restylene were injected into the mid- to deep-dermal layer. Radiesse and Sculptra were injected into the dermal/subcutaneous interface or into the subcutaneous tissue. Silikon 1000 was injected into the subcutaneous tissue. Fillers were injected either immediately preceding or 1 month preceding RF treatment.

The abdominal region was shaved prior to monopolar RF treatment. Treatments were performed using a Thermacool TC System and a 1.5 cm² medium treatment tip at 130 and 162 J (levels 65.0 and 67.0, respectively). A return electrode was placed on a shaved region of the lower back. RF was applied to each treatment area three times (“3 passes”) with a minimum of 2 minutes elapsed time between passes. Relatively high treatment levels were chosen in order to enable the detection of any potential adverse effects of RF treatment over the fillers. Commercial pre-programmed settings were used which consisted of a sequential 0.2-second pre-cool of the skin surface, RF energy delivery over 1 second with concurrent cryogen cooling, and a final 0.7-second post-cool period. Tissue responses were recorded after injection and/or treatment. Injected and/or treated tissue areas were biopsied 5 days, 2 weeks, 1, 2, or 4 month(s) after injection and/or RF treatment. Biopsies for each condition were collected in duplicate. Formalin-fixed, paraffin-embedded specimens were sectioned for hematoxylin–eosin (H&E), and Mason’s trichrome staining using standard methods. Radiesse-filled specimens were decalcified in the paraffin block prior to sectioning. Histological specimens were examined by a board-certified dermatopathologist. Tissue sections were evaluated for inflammatory responses, collagen responses and filler presence. The percentage of the filled area occupied by inflammatory cells was estimated. The intensity of the inflammatory response was graded using the following scale: (0) none; (1) minimal; (2) mild; (3) moderate; and (4) marked. Foreign body response was graded using a modification of the Duranti et al. grading system: (0) no visible reaction; (1) slight reaction with few inflammatory cells; (2) clear inflammatory reaction with few giant cells; (3) granuloma around implants and clear foreign body reaction; and (4) fibrosis with or without foreign body reaction or inflammatory cells [18]. The magnitude of fibroplasia and collagen deposition in and around the filled areas were graded together using the following scale: (0) none; (1) minimal; (2) mild; (3) moderate; and (4) marked. Statistical analysis required larger data sets to achieve significance, therefore data collected at different time points, at both treatment levels, or with and without RF treatment were pooled for analysis as indicated in the text. Statistical analysis was performed using a two-tailed t-test, assuming equal variance.

RESULTS

Time of Treatment Observations

The treatment levels used in this study were higher than levels typically used in the clinical setting. These relatively high treatment levels were selected to magnify any possible adverse effects of RF treatment over fillers. Immediate tissue responses to RF treatment over fillers were monitored. RF treatment over all five fillers injected either immediately before or 1 month prior to treatment produced no incidence of epidermal burns, no increase in observed erythema, and no increase in observed histological effects compared with RF-treated tissue in the absence of filler.

Histological Response

Each of the filler types had a characteristic inflammatory response. Cosmoplast was readily discerned from native collagen because the implant stained lighter in color than native collagen and was not birefringent. Cosmoplast-injected areas showed the presence of lymphocytes, histiocytes and macrophages primarily around the periphery of the implant (Fig. 1A). Giant cells were observed as early as 5 days after injection but were rare. Fibroblasts and vascular channels were present in the interior of the implant.

Silikon 1000 was encircled by a monolayer of macrophages with occasional giant cells by 2 weeks after injection. Some macrophages contained vacuoles or bubbly droplets of phagocytic material. Slight fibroplasia and collagen around the periphery of the silicone droplet was present at 4 weeks (Fig. 1B). Figure 1B represents the greatest extent of fibrous capsule formation associated with silicone observed in this study. No additional increase in collagen around the implant was observed during the 4-month study period.

Radiesse-injected areas contained lymphocytes and histiocytes with giant cells encircling the periphery of the implant at 5 days post-injection. Macrophages appeared to contain small calcium hydroxylapatite particles, which were visible with incomplete decalcification. After 2 weeks, giant cells appeared to be engulfing Radiesse spheres (Fig. 1C). This is not in agreement with the observations of the Lemperle et al. human study, in which Radiesse catabolism was proposed to be enzymatic since macrophages and giant cells were not observed in the implant [14]. By 4 weeks in the present study, focal fibrosis was observed around and within the implant accompanied by an intense giant cell response. At 4 months post-injection, there appeared to be more collagen present at the implant site in the RF treated tissues (Fig. 2A,B).

At 5 days post-injection, the Restylane implant was surrounded by giant cells (Fig. 1D). Lymphocytes and histiocytes were present to a lesser degree. Eosinophils were also observed around the implant periphery with an accompanying eosinophilic and lymphohistiocytic response in the upper reticular dermis. With RF treatment, there appeared to be a lesser inflammatory response at the 5-days-post-injection time point. Fewer eosinophils were...
Fig. 1. Inflammatory response and appearance of injected filler materials in tissue (hematoxylin–eosin (H&E)). A: Cosmoplast at 1 month post-injection without radiofrequency (RF) treatment. *, indicates Cosmoplast. #, indicates native dermal collagen. Bar = 250 µm. B: Silikon 1000 at 1 month post-injection without RF treatment. *, indicates injected silicone droplets. Bar = 250 µm. C: Radiesse at 2 weeks post-injection without RF treatment. *, indicates some of the Radiesse spheres present in the tissue section image. #, shows a Radiesse sphere engulfed by a multinucleate giant cell. Bar = 100 µm. D: Restylane at 2 weeks post-injection without RF treatment. *, indicates some of the Restylane-filled regions. Bar = 250 µm. E: Sculptra at 4 weeks post-injection without RF treatment. *, indicates some of the Sculptra particles present in the tissue section image. Bar = 100 µm.
observed 2 weeks after injection although inflammation in the upper to mid-dermis was still present. With RF treatment, an increase in fibroplasia was observed at 2 weeks post-injection (Fig. 2C,D). At 4 weeks, focal fibrosis around the implant was observed. By 4 months post-injection, the inflammatory response around the remaining implant was mild with few giant cells.

Sculptra-injected areas contained elliptical birefringent particles. Lymphocytes, histiocytes, eosinophils, macrophages, and giant cells were present at the periphery of the implant 5 days after injection (Fig. 1E). Fibroplasia was observed around the implant 2 weeks after injection. At this time-point eosinophils were reduced in number. Fibroplasia was present at the periphery and in the interior of the implant at 4 weeks post-injection. Fibroplasia appeared to be more pronounced in some of the RF treated Sculptra-injected tissues (Fig. 2E,F). The inflammatory response at 4 weeks consisted of predominantly giant cells. By 4 months post-injection, filler presence and fibroplasia were reduced.

In order to assess whether there was a difference in histological tissue response between filler injected immediately before RF treatment and filler injected 1 month prior to RF treatment, we compared three test groups where filler substances were present 2 months after injection. The three test groups were: filler injected and biopsied 2 months later (no RF treatment), filler injected...
immediately before RF treatment and biopsied 2 months later, and filler injected 1 month prior to RF treatment and biopsied 1 month after RF (2 months total elapsed time). Cosmoplast did not persist in the tissue after 2 months and was, therefore, not included in this comparison. In the histologic assessments described (foreign body response, intensity of inflammation, percent inflammation), there was no apparent difference in histological observations between filler injected immediately preceding or 1 month prior to RF treatment. Therefore, tissue specimens treated 1 month after injections were excluded from further analysis. The results below describe the results from tissues treated immediately after injection and injected tissues without RF treatment. Data from the tissues of both animals were pooled together for analysis since no histological differences were apparent between the two animals.

The character of the foreign body response around the fillers was graded using a modification of the Duranti et al. grading system [18]. Restylane [average score of 3.0 (standard deviation, SD = 0.5)], Radiesse [3.7 (0.5)], and Sculptra [3.3 (0.5)] were observed to have a more pronounced foreign body response with giant cell involvement and fibrosis compared to the milder reactions elicited by Cosmoplast [1.7 (0.5)] or Silikon 1000 [2.1 (0.8)]. Specimens with and without RF at time points 5 days post-injection and beyond were pooled for analysis. The difference in foreign body response between the two groups (Restylane, Radiesse, and Sculptra versus Cosmoplast and Silikon 1000) was statistically significant (P<0.05). In addition, there was a statistically significant difference between the foreign body response for Restylane and Radiesse (P = 0.002). The foreign body response in the filled region with monopolar RF treatment was not significantly different from untreated implant for any of the filler types examined.

The percentage of the filled areas occupied by inflammatory cells was estimated and the severity of the inflammatory response for each filler type was scored. In order to perform statistical analysis, inflammatory responses from Restylane, Cosmoplast, Radiesse, and Sculptra at all time-points greater than zero were pooled. Silikon 1000 was excluded from analysis because its inflammatory response was minimal during the study period and did not invade the filler substance. Neither the percentage of the filled areas occupied by inflammatory cells (no RF = 36.4% (SD = 31.5) or with RF = 40.3% (SD 28.2)) nor the severity of the inflammatory response (no RF = 2.9 (SD = 0.6) or with RF = 2.9 (SD 0.7)) was significantly different with or without RF treatment (P = 0.434 and P = 0.902, respectively).

Collagen Response

In the initial histological review described above, RF appeared to increase fibroplasia and the collagen response with three of the fillers (Restylane, Sculptra, and Radiesse). The fibroplasia and collagen deposition responses in and around the filled tissue areas for these three fillers were graded using the following scale: (0) none; (1) minimal; (2) mild; (3) moderate; and (4) marked. The dermatopathologist was blinded to the treatment modality for this appraisal.

Data from RF treatments at levels 65.0 and 67.0 were pooled for analysis since the different treatment levels did not appear to affect histological response or score. No trend or statistically significant change in fibroplasia or collagen response in RF-treated Restylane-filled tissue was revealed when responses were graded (not shown). In the first month after RF treatment over Sculptra-filled tissue, there was a trend toward increased collagen response with RF treatment [average score = 2.4 (SD = 0.5)] versus without RF [2.0 (0.6)] (Fig. 3A). The difference was not significant (P = 0.117). Small sample number is likely to have contributed to the inability to achieve statistical significance with these small changes. In Radiesse-filled areas, there was a trend toward increased collagen response with RF treatment and over the study period (Fig. 3B). Changes at 112 days were determined to be
When all time points greater than 5 days were pooled for analysis, the collagen response in the Radiesse-filled tissue with RF [average score = 2.1 (SD = 0.4)] versus without RF [1.4 (0.4)] was statistically significant ($P = 0.0007$) and changes at 112 days were significant ($P = 0.001$).

**Filler Longevity**

Histological specimens from injected areas were evaluated for the presence or absence of filler. The medium to long-term fillers Radiesse and Sculptra were present throughout the 4-month study period although particles were reduced in size and number at later time points. The permanent filler Silikon 1000 was present throughout the 4-month study period. Monopolar RF treatment had no observable negative effect on the persistence of these fillers in the tissue (Fig. 4A–C). Filler was not observed in some tissue specimens (example in Fig. 4A: Radiesse, with RF at 8 weeks). Since later time points did show the presence of filler, we believe this transient decrease in some samples does not reflect a significant biological event. Additional studies in which filler presence is monitored at time points greater than 4 months and in which larger data sets are collected are required to more accurately assess the residence time of Radiesse, Sculptra, and Silikon 1000 in this animal model.

**Fig. 3.** Collagen response scores for Sculptra (A) and Radiesse (B) with or without RF treatment. Bars represent standard deviations. In instances where there is no bar either $n = 1$ (Sculpta without RF 5, 14, and 112 days; Radiesse without RF 5 and 14 days) or SD = 0 (Radiesse with RF 56 and 112 days). For Radiesse (B), changes at 28 days were not quite significant ($P = 0.075$) and changes at 112 days were significant ($P = 0.001$).

**Fig. 4.** RF effect on filler longevity: (A) Radiesse, (B) Sculptra, (C) Silikon 1000, (D) Restylane, and (E) Cosmoplast. Data are represented as percent injected areas with filler present, where 100% signifies that all histological specimens examined had implant present.
RF treatment had no observable effect on the persistence of the temporary fillers Restylane and Cosmoplast (Fig. 4D,E). After 4 months, Restylane was present in half of the tissue specimens either with or without RF treatment. Cosmoplast was absent from both the untreated and the RF-treated tissue specimens after 2 months. Injectable collagens can produce skin hypersensitivity when they are used cross-species. The relatively short timeframe of Cosmoplast longevity in this study may be a result of a cross-species reaction of the pig to the human-derived collagen. However, no skin reactions were observed in either pig after repeated injections of Cosmoplast over 1½ or 4 months. An alternative explanation is that soft-tissue fillers may be reabsorbed more readily in the juvenile pig abdomen compared to the adult human face. If proven, this difference may provide an experimental advantage when effect on longevity in dermal tissues is examined.

DISCUSSION

Time of Treatment Observations

Prior to this study, it was not known whether RF applied directly over tissue treated with dermal fillers would have an effect on the implant or the surrounding tissue. It was speculated that the implanted substance could significantly change the conductive properties of the tissue either increasing or decreasing its conductivity and the amount of heat produced. We monitored immediate skin responses and saw no adverse effect of RF treatment over tissue injected with the filler substances tested in this animal study. Thermage does not recommend treating over tissue injected with lidocaine or similar anesthetics since these solutions will change the electrical resistance of the tissue and alter the tissue heating profile in an unpredictable way. In addition, patient feedback is needed to assist physicians in selecting appropriate treatment levels.

Histological Responses

All filler substances cause foreign body reactions that may develop into a foreign body granuloma in selected patients [19–23]. This event is less frequent with resorbable filler materials compared to long-lasting implants. In this study, Radiesse and Sculptra, and Restylane to a lesser degree, promoted a foreign body response with giant cell involvement and fibrosis. Inflammatory responses associated with Cosmoplast or Silikon 1000 were mild. The degree of foreign body reaction observed in this study is roughly consistent with the longevity of the filler substances, with the exception of silicone, a permanent filler, which may not develop a foreign body granulomatous reaction until 5–20 years after injection [24–27]. The intensity and character of the inflammatory reaction with Silikon 1000 in this study is similar to that observed by Lemperle [14] although a lower viscosity silicone oil (PMS 350) was used in that study.

No published histological studies are available for Cosmoplast, however, information regarding human placental collagen and cross-linked bovine collagen (Zyplast) is available for comparison. Human placental collagen injected in human skin has been shown to be non-antigenic, eliciting only mild inflammation even in patients with confirmed sensitivities to bovine collagen [10]. Zyplast was FDA-approved in 1985 and has been widely used over the past 20 years. Histological observations of inflammatory reactions elicited by bovine collagen injected in humans generally indicate mild to moderate inflammation characterized by fibroblasts and macrophages predominantly at the periphery of the implanted material, similar to our observations [14,28,29].

Restylane has been observed by others to be free from fibrosis and foreign body reaction and has been shown to persist up to 9 months in injected volar forearm [14,18]. This animal study differs somewhat from the aforementioned studies in that some giant cell involvement and fibroplasia was observed in the Restylane-injected tissues. Like silicone, hydroxylapatite is purported to promote collagenesis around the implant, thus adding volume and utilizing the body’s response to augment tissue [8,30]. In soft tissue laryngeal implantation in rabbits, hydroxylapatite promoted a limited acute inflammatory response and fibrosis with rare giant cells [13]. This response is reported to be dependent on particle shape, with the sharper-edged hydroxylapatite particles having a more pronounced and persistent giant cell response than particles with smooth edges [31]. In human volar forearm, macrophage presence was relatively low, and Radiesse resorption was proposed to occur via enzymatic degradation [14]. This is in contrast to our observations in pig skin where calcium hydroxylapatite particles appeared to be present in macrophages and multinucleated giant cells, suggesting that phagocytosis plays a role in Radiesse absorption.

Polylactic acid particles (Sculptra) have been reported to act as the nucleation site for fibroplasia [8]. Degradation of polymer is associated with an increase in collagen deposition and this may be a consequence of the release of free lactate into the tissue which itself stimulates collagenesis [8,12]. Foreign body inflammatory granuloma formation after implantation of NewFill (Sculptra) has been reported (reviewed in Ref. [32]). This is consistent with our observation of a confluent giant cell response associated with the Sculptra implant.

Collagen Response

Collagen and Restylane augment tissue primarily by adding volumes. Other fillers act as a focus for collagen synthesis (Sculptra), a foreign body that elicits fibrous capsule formation (Silikon 1000), or a scaffold for tissue ingrowth (Radiesse) [8]. It is not unexpected that the combination of Radiesse, which promotes collagenesis, and monopolar RF treatment, which promotes collagen deposition by virtue of its elicited mild wound-healing response, together promote a larger collagen response than filler alone. Our initial histological observations suggested that RF treatment was associated with an increase in fibroplasia and collagen with three of the fillers tested: Restylane, Sculptra, and Radiesse. The tissue samples with these fillers were subsequently subjected to a blinded evaluation of collagen response. This analysis revealed a
statistically significant trend toward increased collagen with RF treatment and Radiesse over the duration of the study period. The trend of increased collagen production with Sculptra treated with RF was not statistically significant. However, we cannot conclude that RF does not affect the Sculptra-associated collagen response since the data set was too small to conclusively determine that the small changes observed were due to chance. A study with more biological replicates would be required to rigorously test the effect of RF on collagenesis.  

**Filler Longevity**  
Radiesse, Sculptra, and Silikon 1000 were present throughout the 4 months study period. Monopolar RF treatment had no observable effect on the persistence of these fillers in the tissue. Additional studies in which filler presence is monitored at time points greater than 4 months and in which larger data sets are collected are required to more accurately assess the residence time of Radiesse, Sculptra, and Silikon 1000 in this animal model.  

RF treatment had no observable effect on the persistence of the temporary fillers Restylane and Cosmostat. It is encouraging that the RF treatment was not observed to affect implant residence time with the fillers tested which vary significantly with respect to their constituent materials and, presumably, thermal labilites. It is known that collagen denaturation occurs at approximately 52–60 °C. Although in vivo temperatures have not been determined during RF delivery using the ThermaCool device, it is estimated that treatment heats tissue to between 50 and 65 °C. The results presented here suggest that the filler substances tested are not de-stabilized by the temperatures achieved during RF treatment using this device. Consistent with this finding, Semchysyn and Kilmer examined whether monopolar RF treatment after botulinum toxin injection affected toxin efficacy and found no adverse effect of tissue heating [33].  

**Implications**  
The results from this study suggest that multiple passes of monopolar RF treatment directly over filler-injected skin does not promote immediate adverse tissue responses nor does it adversely affect the residence time of the various fillers. In addition, RF treatment was observed to enhance collagen deposition within and surrounding the filler-injected areas. Larger studies are required to rigorously test the effect of RF on collagenesis in association with fillers. This animal model does not afford an assessment of the cosmetic clinical outcome of combining soft-tissue filler augmentation with monopolar RF treatment, which will require human studies.  

**REFERENCES**  