Objective: To evaluate histologically the extent and character of tissue in-growth after injection of calcium hydroxylapatite for soft-tissue augmentation.

Methods: Prospective case series of 8 patients from a private facial plastic surgery practice. Each patient was injected subdermally between the dermis and the subcutaneous fat with 0.1 mL of calcium hydroxylapatite in the superior postauricular sulcus. One month later, 2 patients underwent excision of the injected material, which was submitted for routine histological evaluation by an independent dermatopathologist. The material was stained with hematoxylin-eosin and trichrome to assess new collagen deposition. One of these patients was reinjected in the contralateral postauricular sulcus. Six and 18 months after injection, 3 patients and 1 patient, respectively, underwent excision of the injected material, which was then submitted for histological evaluation of the degree and character of tissue in-growth and tissue reaction.

Results: The calcium hydroxylapatite particles were found to be surrounded by a lymphocytic infiltrate with multiple foreign body giant cells. There was no evidence of new collagen formation, migration, or heterotopic bone growth. Two patients were lost to follow-up.

Conclusions: Calcium hydroxylapatite is a stable soft-tissue filler that stimulates an immune response with lymphocytic infiltration and foreign body giant cell formation. We found no evidence of new collagen formation 1, 6, or 18 months after injection.

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Soft-tissue augmentation of the face has been attempted for many years using a variety of substances. Inorganic substances have included silicone gel, polyethylene, polycrylamide gel, and expanded polytetrafluoroethylene. Problems with these substances have included extrusion, severe granuloma formation, and palpability. Organic substances have included mica, ivory, liquid paraffin, and coral. More recently, human and bovine collagen have been used with variable success. Limitations have included loss of volume over a relatively short period and the potential for allergic reactions in up to 3% of patients. Naturally occurring fillers, including hyaluronic acid, poly-L-lactic acid, and autologous fat, are becoming more popular. However, none of these substances has proved to be the ideal injectable material. Migration, host immunological responses, absorption, and rejection have been the biggest hurdles. The ideal implant should be noncarcinogenic, nonteratogenic, nontoxic, nonimmunogenic (no foreign body or inflammatory reaction), nonresorbable, easy to work with, and malleable. The material should have a tactile feel similar to that of tissue, have a low or zero extrusion rate, allow biointegration of the implant with the surrounding tissue, and be cost-effective. Attempts to reach these goals have led to the introduction of calcium hydroxylapatite (CaHA) (also referred to as calcium hydroxyapatite).

Synthetic CaHA (Radiesse; BioForm Medical Inc, San Mateo, California) was approved by the US Food and Drug Administration (FDA) Center for Devices and Radiological Health for augmentation of the vocal fold, radiological marking of soft tissue, and augmentation of dental intraosseous and oral/maxillofacial defects including craniofacial defects. On December 28, 2006, the FDA approved Radiesse for the correction of moderate to severe facial wrinkles and folds and for the correction of facial lipoatrophy in people with human immunodeficiency virus. The formulation consists of 35% spherical particles of synthetic CaHA blended in an
Figure 1. Calcium hydroxyapatite spherules on high-power magnification without staining (original magnification ×200). The spherules are fairly uniform in size and shape.

Figure 2. Patient 1. One month after injection with calcium hydroxyapatite (CaHA), high-power light microscopy shows a few CaHA spherules surrounded by numerous histiocytes and a few foreign body giant cells (arrow) (hematoxylin-eosin, original magnification ×400).

aqueous gel carrier that contains water, glycerin, and sodium carboxymethylcellulose. The CaHA particles range from 25 to 45 µm in diameter. Synthetic CaHA is a standard biomaterial (American Society for Testing and Materials F1185) that is identical in chemical composition to the inorganic constituent of teeth and bones. The gel ingredients are US Pharmacopeia–grade pharmaceutical excipients that are classified “generally recognized as safe” by the FDA.

Because CaHA is the major mineral component of bone, it should not provoke an immune-system reaction. However, concern for heterotopic bone growth has been raised. Previous animal studies using CaHA have noted that heterotopic bone growth did not appear to develop when particles were placed in soft tissues away from periosteum. Drobeck et al. studied subcutaneous implantation of CaHA in rats for up to 1 year and in dogs for up to 6 years. No evidence of heterotopic bone formation, migration, or chronic inflammation was noted.

A study by Marmur et al. proposed that CaHA stimulates the formation of new collagen around the spherules, which appeared 6 months after injection in that study. The purpose of the present study was to demonstrate the local soft-tissue reaction to subdermally injected CaHA in humans.

METHODS

Informed consent was obtained from 8 patients of a private facial plastic surgery practice. Exclusion criteria included pregnancy, infection or inflammation at the injection site, surgery at the injection site within the preceding 12 months, previous reaction to any soft-tissue filler, immunodeficiency disorder, or current therapy with immunomodulating medication. Each patient was injected subdermally between the dermis and the subcutaneous fat with 0.1 mL of CaHA in the superior postauricular sulcus. One month later, 2 patients underwent excision of the injected material and surrounding soft tissue, which was submitted for routine histological evaluation by an independent dermatopathologist (D.S.B.). All specimens were stained with hematoxylin-eosin and trichrome to assess new collagen formation. One of these patients was reinjected with CaHA in the contralateral postauricular sulcus. Six months after injection, 3 patients underwent excision of the injected material and surrounding soft tissue, which was sent for histological evaluation. Eighteen months after injection, 1 patient underwent excision of the injected material and surrounding soft tissue, which was also submitted for histological evaluation. The 6- and 18-month biopsy specimens were analyzed for the degree and character of tissue in-growth, tissue reaction, and/or ossification.

RESULTS

A smear was performed on a blank slide of the CaHA spherules in their aqueous carrier prior to injection (Figure 1). The spherules were noted to be uniform in size and shape under light microscopy.

Light microscopy of the tissue samples 1 month after injection showed the CaHA spherules surrounded by a significant foreign body reaction. Numerous histiocytes and a few foreign body giant cells were noted (Figure 2). There was no noticeable external skin change or inflammation. There was no evidence of ossification, and trichrome staining did not reveal new collagen formation. The CaHA spherules did not change significantly in size or quality.

The samples acquired 6 months after injection were noted to also have a significant foreign body reaction. Light microscopy showed intact CaHA spherules within the native soft tissue, surrounded by a dense population of histiocytes, foreign body giant cells, and fibroblasts (Figure 3). The foreign body giant cells were seen to engulf many of the spherules (Figure 4). There was no sign of ossification, and trichrome staining did not reveal new collagen formation. Again, no external signs of inflammation were noted on the skin, and there were no palpable nodules. The CaHA spherules did not change significantly in size or quality.

Because 2 patients were unavailable for follow-up, a single sample was acquired 18 months after injection. That sample was noted to have the same degree and type of foreign body reaction as the previous specimens. Light microscopy showed a few CaHA spherules surrounded by histiocytes and foreign body giant cells (Figure 5). Again, there was no evidence of collagen deposition on trichrome staining and no sign of ossification. No external
signs of inflammation were noted on the skin. The CaHA spherules did not change significantly in size or quality.

**COMMENT**

Lemperle et al\(^9\) studied the histological reactions and persistence of various fillers placed into the human forearm. Of note, CaHA microspheres induced almost no foreign body reaction in the forearm but were absorbed by the skin 12 months after implantation. Other fillers, including collagen, hyaluronic acid, and dextran microspheres, induced more aggressive foreign body reactions and disappeared more quickly.\(^9\) In contrast, Misiek et al\(^10\) studied the implantation of hydroxylapatite particles in dogs and discovered a significant foreign body reaction, with granulation tissue that subsided over several months.

In the present study, an aggressive foreign body reaction was noted with no evidence of new collagen formation. This response did not subside during the 18-month study. Because of the nonimmunogenic nature of CaHA, this could be a response to the gel carrier or represent distension of the tissues. This finding is in contrast to those of Lemperle et al\(^9\), who noted that the entire implant was surrounded by a fine fibrous capsule and that single microspheres were encapsulated by a thin fibroblastic stroma with flattened cells. Few macrophages were seen in their study, suggesting that CaHA microspheres are degraded by enzymatic breakdown rather than by phagocytosis. Marmur et al\(^8\) also demonstrated fibroelastic replacement of the aqueous gel carrier in a study similar to this one. Their findings showed collagen surrounding the microspheres 6 months after injection. Our study found no evidence of new collagen deposition in samples obtained 1, 6, or 18 months after injection and stained with trichrome. The contrast in our findings to those of previous investigators could be a result of the small sample size. Further studies with a much larger sample size could provide more definitive information on the ability of CaHA fillers to stimulate collagen formation and/or an inflammatory response.

Stein et al\(^4\) noted osseous metaplasia after injection of hydroxylapatite cement into the vocal folds of dogs, with minimal inflammatory reaction and no migration of the cement to the regional lymph nodes. No osseous metaplasia was noted in the human samples analyzed in the present study.

The results of this study could have been improved by the inclusion of a larger number of study subjects, extension of the study period beyond 18 months, and placement of the material in commonly augmented sites (ie, the melolabial folds). Longer follow-up may have demonstrated replacement of the inflammatory infiltrate with new collagen. However, the goal of this study was to determine the histopathologic response of the host tissue to local injection of CaHA. Our findings indicate a significant foreign body reaction and chronic inflammatory response to the injected material. This response contradicts reports of the inert properties of CaHA. However, these findings may help explain the nonpermanence of the augmentation afforded by CaHA. The host’s inflammatory response may hasten the breakdown of the microspheres, shortening the proposed duration of augmentation.

In conclusion, CaHA appears to be an effective and safe injectable filler. Calcium hydroxylapatite possesses many of the characteristics of the ideal filler. The mate-

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**Figure 3.** Patient 2. Six months after injection with calcium hydroxylapatite (CaHA), high-power light microscopy shows numerous CaHA spherules surrounded by a dense population of histiocytes, foreign body giant cells, and fibroblasts (hematoxylin-eosin, original magnification \(\times 400\)).

**Figure 4.** Patient 3. Six months after injection with calcium hydroxylapatite (CaHA), high-power light microscopy shows scattered CaHA spherules surrounded by numerous histiocytes and foreign body giant cells. Foreign body giant cells were noted engulfing spherules (arrows) (hematoxylin-eosin, original magnification \(\times 400\)).

**Figure 5.** Patient 4. Eighteen months after injection with calcium hydroxylapatite (CaHA), high-power light microscopy shows a few CaHA spherules surrounded by histiocytes, fibroblasts, and foreign body giant cells.
Material is noncarcinogenic, nontoxic, easy to work with, malleable, relatively long-lasting, and somewhat removable. However, the material does cause an immunologic response, which most likely contributes to its variable resorption rate.

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Correction

Unreported Financial Disclosure: In the Research Let-
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