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Clinical, histologic and electron microscopic findings after injection of a calcium hydroxylapatite filler

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BACKGROUND: Calcium hydroxylapatite (CaHa) is one of many newly available soft tissue fillers.
OBJECTIVE: We have, in this pilot study, evaluated the clinical, histologic and electron microscopic ultrastructural changes seen with CaHa at 1 and 6 months after skin injection.
METHODS: Each of the three subjects was injected in the postauricular area with 0.1 cc of CaHa gel. A 3-mm punch tissue biopsy was taken at 1 and 6 months post-injection. Biopsies were analyzed by histopathology and electron microscopy. Clinical results after injection of the nasolabial folds were also evaluated.
RESULTS: CaHa particles were found to persist at 6 months with evidence of new collagen formation being seen. Patients still showed clinical improvement at this time.
CONCLUSION: This study is the first in vivo ultrastructural analysis of the biologic response to CaHa in human skin. CaHa shows clinical, histologic and electron microscopic evidence of persistence at 6 months. *J Cosmet Laser Ther* 2004; **6**: 223–226

Introduction

Soft tissue augmentation has become an enormously popular procedure over the past two decades. As a result, increasing numbers of filler agents have become available. Calcium hydroxylapatite (CaHa) injections represent one of these promising new soft tissue fillers.

Fillers are generally classified into four major types: synthetic, xenogeneic, homogeneic, and autogeneic.[1] Synthetic fillers include silicone, poly-methylmethacrylate (PMMA) beads and now CaHa (Radiesse; Bioform, Franksville, WI, USA). Xenograft fillers (donor and recipient from different species) include bovine collagen and hyaluronic acid derivatives. Homogeneic fillers (donor and recipient from the same species) include those agents derived from accredited tissue bank human cadaveric tissue. Autogeneic materials (donor and recipient from the same individual) include autologous fat, and autologous collagen and/or dermal fibroblasts.

An ideal filler is one which is biocompatible with human tissue, inert, durable, easy to inject, and does not require over or undercorrection.[2] In order to understand the human skin biologic response to CaHa, we performed a pilot study evaluating the clinical, histologic and clinical ultrastructural changes seen at 1 and 6 months after human skin injection.[3,4]

Materials and methods

Three female subjects were enrolled in the study after signing informed consent. Exclusion criteria included prior soft tissue filler implant injection into the treatment sites, active inflammation, pregnancy, and immunodeficiency disorders and/or medication that might obscure the inflammatory response. Each of the three subjects was injected in the postauricular area with 0.1 cc of CaHa gel. The CaHa gel consisted of calcium and phosphate ions in a gel-based aqueous formulation of sodium carboxymethyl cellulose, glycerin, and high purity water. The post-injection site in the postauricular area initially appeared as a 1-cm dermal nodule. This site was measured and photographed to ensure accurate placement of the tissue biopsies at follow-up visits.

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Each subject was also injected in the bilateral nasolabial folds with 0.5 cc of CaHa gel to each fold. Punch tissue biopsies of 3 mm were taken from the postauricular site at 1 and 6 months after the injections. The tissue specimen was then bisected so that one piece was placed in formalin and the other in glutaraldehyde. Biopsies were analyzed by histopathology and electron microscopy for inflammatory cell reaction, fibrosis, ossification, and/or granuloma formation. Clinical results were evaluated by two non-treating physician observers by way of digital photography. Subjects at 6 months were asked if they were very satisfied, moderately satisfied, minimally satisfied or unsatisfied with their clinical results. Objective independent 6-month digital photograph improvement was categorized as significant, moderate, mild or none.

Results

All three treated subjects reported that they were 'very satisfied' with the clinical results at 6 months after nasolabial fold injection. The treated areas were soft and natural to feel. No patients reported nodules, redness, bruising, extrusion of gel material, or infection. Both physician observers noted moderate to significant clinical improvement in the nasolabial folds of each treatment patient (Figures 1 and 2).

Standard light microscopy of tissue specimens at 1 month post-injection show microspherules of CaHa gel with scant or no inflammatory cell response or fibrosis (Figures 3 and 4). The CaHa microspherules were distributed freely in the junction between the dermis and subcutis. They appear as smooth, slightly irregular, small pink spherules, measuring 35 mm on average. The minimal early inflammatory response is largely composed of histiocytes.

Electron microscopic sections of biopsies taken 1 month after treatment show findings consistent with those of light microscopy (Figure 5). The CaHa microspherules are scattered in the dermal/subcutaneous junction surrounded by pre-existing Type I collagen and scant inflammation.

Light microscopic sections at 6 months showed tight aggregates of microspherules with a fibroelastic replacement

Figure 1
Pre-injection clinical photograph of nasolabial folds.

Figure 2
Six months post-injection clinical photograph of nasolabial folds.
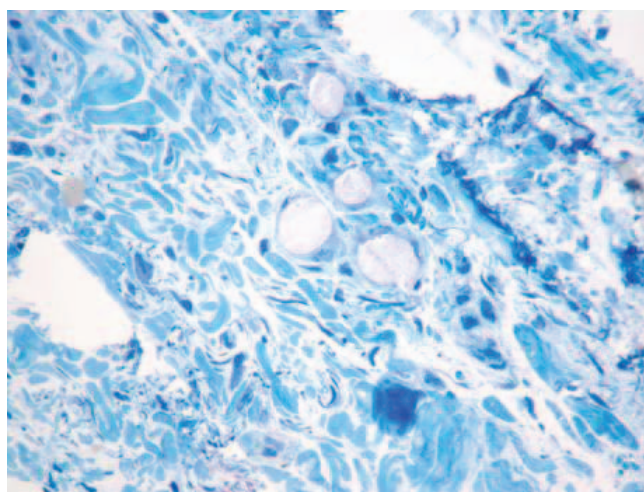

Figure 3
Thick section light microscopy at 1 month post-injection showing a microspherule engulfed by a histiocyte.
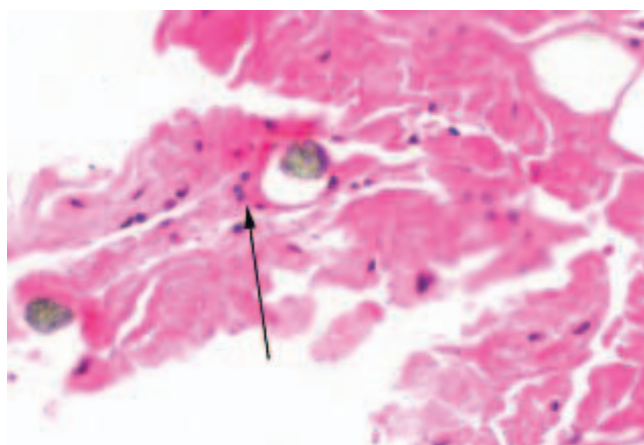

Figure 4
Light microscopic section at 1 month post-injection showing micro-spherules at the dermal subcuticular junction and a slight increase in histiocytes (arrows).

of the aqueous gel. Multinucleated giant cells (arrow) are seen surrounding each microspherule (Figure 6). No granuloma formation, ossification, or foreign body reactions
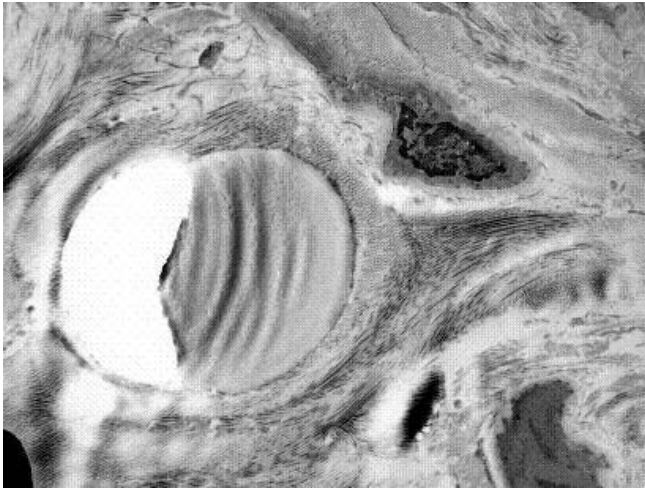
Figure 5
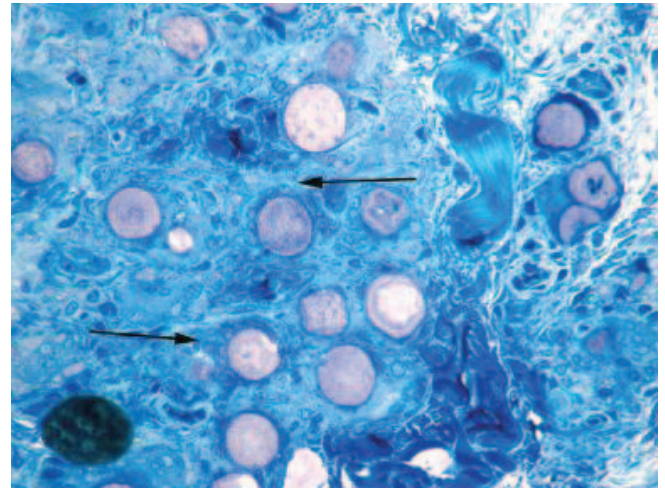*Electron microscopic sections at 1 month showing microspherules and a slight increase in histiocytes.*

Figure 7
Thick section light microscopy at 6 months showing microspherules surrounded by thick collagen and histiocytes (arrows).
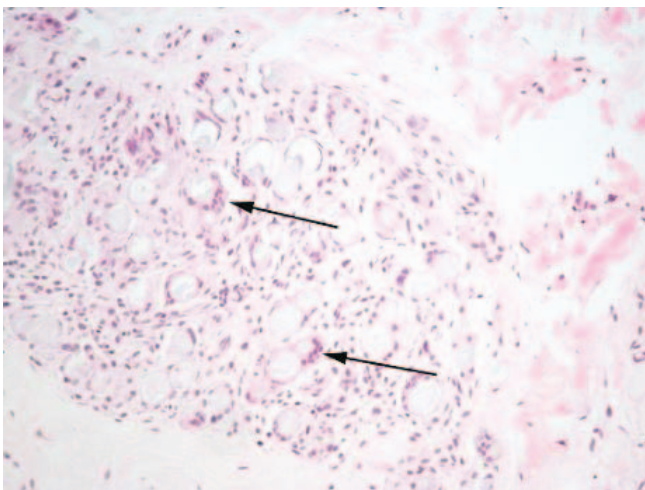

Figure 6
Light microscopy at 6 months showing tight aggregates of microspherules with a fibroelastic replacement of the aqueous gel and multinucleated giant cells (arrow) surrounding each microspherule.

Figure 8
Electron microscopic section at 6 months showing a microspherule engulfed by a tissue macrophage.

are evident. The spherules at 6 months are no longer regular and smooth as in the 1-month specimens. However, the CaHa spherules show no tissue migration, remaining well located in the dermal/subcutaneous junction. Light microscopic sections at 6 months also showed microspherules surrounded by thick collagen and histiocytes (arrows) (Figure 7).

Electron microscopic sections at 6 months reveal intracellular and extracellular material consistent with calcium particles. Microspherules appear to be engulfed by tissue macrophages (Figures 8 and 9).

Discussion

CaHa gel is a new and increasingly popular choice in the field of soft tissue augmentation. It fills a clinical niche for those patients who wish to have a longer-lasting filler, albeit one that is not permanent.[5]

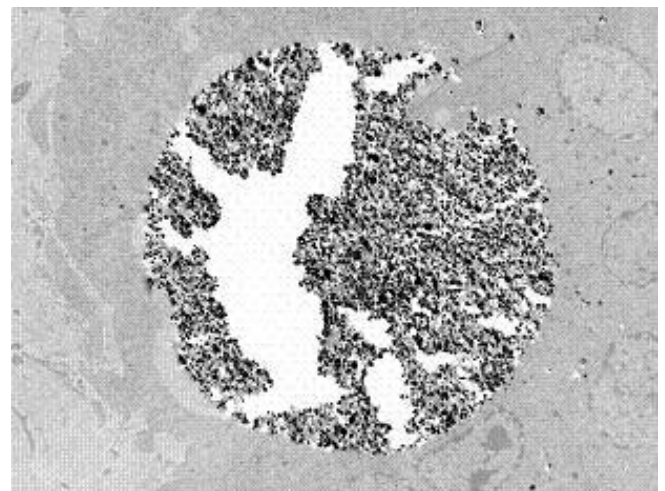For over 25 years CaHa has been used as a synthetic

Figure 9
Electron microscopic section at 6 months showing both an intact microspherule and one undergoing a histiocytic-derived catabolic process into smaller particles of calcium (black particles). The phosphate ions are not seen because they are dissolved in the processing of tissue for electron microscopic analysis.

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implant. It is currently FDA cleared for radiographic tissue marking, vocal fold augmentation, and oral maxillofacial defects.[6–9] Additional studies have also evaluated the role of CaHa for stress urinary incontinence, vesicoureteral reflux, HIV-associated lipoatrophy, cleft lip/cleft palate augmentation, and soft tissue augmentation of the chin and nasolabial folds.[10,11]

CaHa has several characteristics that make it a good soft tissue filler choice. CaHa is composed of 35-mm diameter smooth microspheres suspended in a gel carrier. It is biocompatible with human tissue and safe. The CaHa particles are made of calcium and phosphate ions identical to the mineral portion of bone. CaHa requires no allergy tests, allowing for immediate treatment. It is metabolized through normal homeostatic mechanisms that naturally occur in the body via macrophage phagocytosis similar to degradation of small fragments of bone. It is durable, lasting 2–7 years in vivo radiographically, with a 2-year shelf-life at room temperature. The longevity of clinical results can be expected to vary with each patient.

The characteristics of the CaHa gel carrier are also of note. The gel is an aqueous formulation of sodium carboxymethyl cellulose, glycerin, and high purity water. It has been used as a vehicle for multiple injectable products and is classified as 'generally recognized as safe' by the FDA. It is cohesive, with high viscosity and elasticity, allowing minimal implant leakage to the surrounding tissue planes. In addition, the gel formulation offers a 1:1 implant-to-tissue defect correction allowing physicians to avoid the overcorrection necessary with some fillers.

The biology of CaHa also appears to be different from many fillers. Animal studies have been conducted to evaluate the long-term safety of CaHa implants (personal communication, Bioform Internal Studies). Microscopic analyses of implant sites at serial time points over a 36-month trial period revealed an early macrophage response to the gel followed by a fibrotic response to the microspherules. This early macrophage activity associated with the sodium carboxymethyl cellulose subsides and is replaced by a fibrous encapsulation of individual microspherules.

To our knowledge, our pilot study is the first in vivo ultrastructural analysis of the biological response to CaHa in human skin. Our results are quite similar to those found in animal studies. However, several points are worth mentioning. We found that the early tissue macrophage response to the filler was minimal. Thus, the clinical fill effect was due to the space-occupying characteristics of the synthetic CaHa spherules and its gel carrier. Over time, an increased histiocytic response was seen leading to a notable electron microscopic change in the appearance of the CaHa particles. The particles were more particulate and distributed both intra- and extracellularly. With the passage of time, the increase in histiocytes and associated fibroblasts appears to anchor down the microspherules as well as to induce new collagen formation as the aqueous gel is metabolized. These results were consistent among all three patients studied.

Although animal studies have suggested that CaHa may last 5 years in tissue, our pilot trial in three subjects only suggests that the human response is at least 6 months. In addition, our histologic and electron microscopic findings were seen in the postauricular area. Larger studies are required to determine the response in other human anatomic areas.

Conclusion

Soft tissue augmentation for the treatment of the aging face has undergone a revolution over the past two decades. Our study is the first in vivo ultrastructural analysis of the biological response to CaHa in human skin. It would appear that CaHa is easy to use and shows clinical, histologic and electron microscopic evidence of persistence. Future larger studies will evaluate how long the response to this filler is, in a variety of human anatomic areas.

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