Sterility of Injectable Fillers Containing Nonanimal Stabilized Hyaluronic Acid, Hyaluronic Acid, or Calcium Hydroxylapatite After Long-term Storage

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Injectable fillers containing Restylane (nonanimal stabilized hyaluronic acid, NASHA), Juvéderm (hyaluronic acid, HA), or Radiesse (calcium hydroxylapatite, CaHA) are commonly used for soft tissue augmentation and volume correction. Contents of the syringes often are used on patients and stored for subsequent visits with the same patient, despite manufacturer recommendations to discard unused filler. The primary objective of this study was to determine the incidence of infectious contamination in fillers stored after patient injection. Previously used and stored syringes of NASHA (n=23), HA (n=5), and CaHA (n=4) were obtained from physicians in 2 separate practices. The previously used syringes were stored with clean technique at 2°C and were intended for use within 2 weeks; however, they were never needed again. After being stored for an average of 30, 16, and 27 months, respectively, the remaining contents of the syringes were cultured under aerobic and anaerobic bacteria, acid-fast bacilli, and fungal growth conditions. There was no growth in bacterial, acid-fast bacilli, or fungal culture media. The results of this study support that injectable fillers of NASHA can be stored under clean conditions without evidence of contamination for over 2 years and suggest that HA and CaHA remain free of contamination after lengthy storage as well.

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Restylane, Juvéderm, and Radiesse are popular fillers in the United States. Restylane, a nonanimal stabilized hyaluronic acid (NASHA) gel, and Juvéderm, a cross-linked hyaluronic acid (HA) gel, are both indicated for mid- to deep-dermal implantation for the correction of severe facial wrinkles and folds. Radiesse, a calcium hydroxylapatite (CaHA) gel, is indicated for subdermal implantation to correct lipoatrophy associated with human immunodeficiency virus as well as severe facial wrinkles and folds.[1]

Depending on the volume to be filled, the entire syringe contents may not be required. The remaining filler is sometimes stored for subsequent use in the same patient.

Manufacturers warn against this practice, stating that sterility may be compromised.[2,3] To date, there is no published evidence of an increased complications rate with repeated use of injectable syringes in the same patient.[3,4] However, with the advent of biofilms and during an age of tremendous increase in filler procedures, meticulous techniques and careful procedures are necessary. We designed a retrospective study to investigate whether opened products become contaminated during office use and subsequent storage.

Used syringes were examined for evidence of contamination by infectious entities. Novel aspects of this study are the prolonged storage of NASHA (an average of 30 months compared to 6.5 months in prior studies), testing for fungal and *Mycobacterium* contagion in addition to aerobic and anaerobic bacterial cultures, and the assessment of HA and CaHA for contamination, which has not been previously studied.

Approval for this study was obtained from the Institutional Review Board at Mount Sinai School of Medicine. Thirty-two syringes were collected from 2 separate dermatology practices and tested by the Mount Sinai Laboratories (Table). Both physician practices used clean technique to store and reuse the syringes, using a new sterile needle for each injection. Syringes were recapped with unused needles and stored at 2°C between uses. Specimens collected for this study had been intended for reuse by patients requiring a touch-up within 1 to 2 weeks. The materials that were not used for touch-ups remained in storage and were later collected for testing.

Samples from all syringes were placed on dermatophyte test media and checked twice weekly for 3 weeks before being considered negative for fungal growth. A sample from each syringe also was expressed and transferred to a sterile tube. Each sample was cultured onto a trypticase soy agar plate containing 5% sheep blood, chocolate agar, and MacConkey agar plates. These plates were incubated at 35°C in the presence of 5% carbon dioxide. Plates were examined daily for 10 days before cultures were considered negative for bacterial growth.

The same sample was cultured onto a Middlebrook 7H11 agar plate and 0.5 mL inoculated into a supplemented mycobacteria growth indicator tube (MGIT) to determine the presence of mycobacteria. The Middlebrook 7H11 agar plate was incubated at 35°C in the presence of 5% carbon dioxide for 6 weeks. The MGIT was placed into the BACTEC MGIT 960, where the cultures were continuously monitored for 6 weeks after which time they were considered negative.

There was no growth in any of the fungal, bacterial, or acid-fast bacilli culture media. The lack of bacterial growth suggests that the syringes remained free of biofilm contamination as well. This study extends the previous evidence that NASHA syringes remain free of contamination between patient uses and also confirms, albeit with relatively few syringes, the sterility of HA and CaHA after patient use. Testing for bacterial and fungal contamination is warranted as these pathogens can survive for several months on inanimate surfaces.[4]

In light of evidence supporting minimal risk of retrograde bacterial contamination, manufacturers of NASHA have emphasized the risk of viral contamination.[5] Viral cultures were not performed because of the low

Syringe Data

Filler	Count	Average Storage Period, mo	Range, mo
NASHA	23	29.7	11.5–40.5
HA	5	15.9	10.0–19.0
CaHA	4	26.6	26.0–28.0

Abbreviations: NASHA, nonanimal stabilized hyaluronic acid; HA, hyaluronic acid; CaHA, calcium hydroxylapatite.

likelihood of viral growth without cellular association, especially after lengthy storage. Viruses have been shown to persist for up to 2 months depending on the type of virus, structure, and presence of an organic matrix.[4,6]

Limitations to our study should be noted. First, we used dermatophyte test media rather than a wider range of growth media for fungal culture. Second, we did not test fillers mixed with local anesthetic as these were only recently approved for use. Third, a limited number of syringes were available for testing.

The results of this study demonstrate that injectable fillers of NASHA that were stored under clean conditions for an average of more than 2 years had no evidence of contamination. Furthermore, we have shown that, in this study, other types of fillers, such as HA and CaHA, may be similarly resistant to contamination. Although the products themselves appear to be resistant to contamination, biofilm formation may occur at the point of injection should the needle-tip insert contaminants from the skin surface into the dermis or subcutis.

Meticulous injection techniques by physicians in clean, medical settings are more critical than ever before given the increase in filler procedures and subsequent side effects. Although this study suggests that retrograde contamination in the syringe is less likely than perhaps previously thought, further study would be necessary to better delineate the safety of using stored syringes of filler.

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