Soft Tissue Fillers and Biofilms

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ABSTRACT

The purpose of this study was to review the clinical course of reported hypersensitivity reactions associated with use of soft tissue fillers and the response of these reactions to treatment. In this comprehensive literature review, we identified ~40 published reports that together reported numerous adverse reactions associated with use of nonpermanent soft tissue fillers; however, very few of these reactions were consistent with type I immediate or type IV delayed hypersensitivity reactions. Based on their clinical course and response to treatment, most reported hypersensitivity reactions are likely due to an infectious process. Although there are no confirmed reports of biofilm reactions associated with nonpermanent fillers, the possibility of bacterial contaminants should be considered in acute or chronic inflammatory reactions associated with use of nonpermanent soft tissue fillers. Early treatment with antibiotics is recommended.

KEYWORDS: Filler, complications, biofilm, adverse event, hypersensitivity reaction

Use of cosmetic fillers has grown substantially in recent years. Not surprisingly, reports of complications associated with use of these products have also increased. As a result, soft tissue fillers are falling under greater scrutiny by consumers, physicians, and regulatory agencies. A better understanding of the nature of potential complications associated with use of soft tissue fillers will aid in the prevention and appropriate treatment of adverse effects.

The most commonly used material in nonpermanent soft tissue fillers is hyaluronic acid (HA), a complex carbohydrate found in all animal species. HA is obtained through the fermentation of Streptococcus equi. Prior to 1999, the reported rate of hypersensitivity reactions was 0.07%. After the introduction of a more highly purified product containing lower amounts of protein, the incidence of hypersensitivity reactions decreased to 0.02%. Other commonly used fillers such as calcium hydroxylapatite and poly-L-lactic acid have also been associated with hypersensitivity reactions.

Hypersensitivity reactions occurring after use of nonpermanent filler treatments are poorly understood yet frequently reported in the literature. Classically, hypersensitivity reactions are immune system reactions defined as one of four variations (Table 1). Specifically, type IV reactions involve an inflammatory process where a foreign antigen results in an upregulation of inflammatory mediators, and macrophages and leukocytes are summoned to the site. The antigen is engulfed by the white blood cells and the macrophages, multiple lymphokines are secreted, and the macrophages come together to form a giant cell, which histologically appears as a granulomatous infiltrate.

Often, complicating filler reports are deemed “hypersensitivity reactions,” indicating that an immune-related event occurred whether an immediate IgE-mediated response or a delayed T cell-mediated response.
Table 1  Classical Definitions of Hypersensitivity Reactions

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<th>Coombs-Gell Classification of Hypersensitivity Reactions</th>
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response. However, allergen workups confirming an immune-related event have been less forthcoming. The titles of several reports suggest immediate or delayed immune-mediated reactions after use of HA fillers despite a paucity of documented evidence. Follow-up evaluations to confirm the occurrence of immune-related events are usually negative. These so-called hypersensitivity reactions may be secondary to an infectious process.

At the root of the aforementioned infectious process is the inherent characteristic of bacteria to be incredibly tenacious. Bacteria are arguably the most adaptive organisms known. Some bacteria can survive and thrive under conditions of extreme temperature, pH, desiccation, and radiation. Their ability to continuously adapt and evolve is well known as they become increasingly resistant to growing numbers of antibiotics. Part of their success may be due to their ability to form biofilms, protected complex aggregates in which bacteria adhere to one another and to material surfaces. They do so by secreting an extracellular matrix consisting of multiple carbohydrates, including HA, that serves as a protective barrier. A biofilm is "a microbiologically derived sessile community characterized by cells that are irreversibly attached to a substratum or interface to each other [and] are embedded in a matrix of extracellular polymeric substance that they have produced and exhibit an altered phenotype with respect to growth rate and gene transcription."

Biofilms are difficult to identify because they are slow growing and often produce negative culture results. Fluorescent in situ hybridization (FISH) analysis using peptide nucleic acid probes against bacterial DNA sequences is currently the best way to detect biofilm bacteria. Polymerase chain reaction is less effective because structural genetic changes may hinder epitope targeting. Bacteria that have been identified within biofilms include *Staphylococcus aureus*, *Streptococcus sanguis*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Pseudomonas pseudomallei*, and *Escherichia coli.*

There have been multiple FISH-confirmed reports of biofilm reactions in unapproved forms of permanent soft tissue fillers, but confirmed reactions have yet to be reported in approved nonpermanent fillers currently in use today. It seems that the nonpermanent nature of fillers such as HA, calcium hydroxylapatite, and poly-lactic acid would reduce the risk for biofilm reactions; however, several reports have established the longevity of HA for up to 36 months and calcium hydroxylapatite and poly-lactic acid for up to 5 years. The prolonged duration of these products suggest they may also pose a risk for complications due to biofilms similar to permanent fillers.

The following review was performed to evaluate the nature, treatment, and course of action of reported hypersensitivity reactions associated with use of soft tissue fillers. Particular attention was paid to determine whether the behavior of these reactions is consistent with either infectious or immune-mediated reactions.

LITERATURE REVIEW

A search of the U.S. National Library of Medicine (Bethesda, MD) was performed using the search terms "hyaluronic acid" or "dermal filler" or "soft tissue filler" or "proprietary soft tissue filler brand names" and "complications" or "adverse reactions" or "reactions" or "side effects." The bibliography of each publication was searched, and suitable reports cited were also obtained. All relevant reports published through May 2010 were obtained. Approximately 40 published reports that met the search criteria were obtained and reviewed.

RESULTS

A series of 15 patients was presumed to experience delayed immune-mediated reactions after treatment with HA alone or in combination with acrylic hydrogel. Symptoms consisted primarily of inflammatory nodules. Allergy testing was negative, and none of the patients were administered a rechallenge exposure.

In one report, severe swelling began 10 minutes after the injection of an HA-containing filler. A presumptive diagnosis of acute angioedema was made, and the patient was treated with desloratadine and a tapering course of oral corticosteroids. The swelling resolved over 2 weeks but the patient was lost to further follow-up. Swelling and induration that occurred 3 weeks after treatment with HA was diagnosed as a delayed hypersensitivity reaction. The swelling responded to treatment with oral corticosteroids, but this patient was also lost to follow-up.

De Boulle reported an apparent delayed hypersensitivity reaction occurring 8 weeks after treatment with HA consisting of a full-body macular, urticarial, and popular rash. Although a subsequent intradermal rechallenge on the forearm was negative, the symptoms recurred 1 month after facial retreatment. Only one
report described the return of symptoms after a rechallenge with the product. In that case, an exudative reaction that later became granulomatous occurred 2 days after the injection of an HA product for facial wrinkles. The eczematous reaction peaked in 4 to 6 days and was healed after 10 to 11 days. An intradermal rechallenge with the same product on the forearm resulted in a similar reaction.4

Granuloma formation was associated with products containing HA alone18–22 or in combination with acrylic hydrogel.5,18,19,21,23 In most instances, granulomas occurred 4 to 24 months after treatment19,21–23 although there were several reports of granulomas developing after 2 to 5 weeks.5,20,21 Among the 11 patients described by Lombardi et al, the onset of granulomas ranged from 6 months to 6 years.18 All of these cases were confirmed by biopsy.5,19–23

IgE and IgG antibodies were measured after several instances following typical hypersensitivity reactions, but the results were negative. In a hypersensitive reaction secondary to HA reported 1 month after a retreatment failed to improve with intralesional steroids and antibiotics and only resolved after product removal. Pathology evaluation revealed a histiocytic infiltrate. Intradermal skin testing performed after resolution showed no evidence of inflammatory response for the next 28 days. The authors suggested an infectious origin responsible for the hypersensitivity reaction.24

There was one report of a biopsy-proven foreign body granulomatous reaction after calcium hydroxylapatite injection, but immunologic testing was not performed.25 The rate of palpable nodules after use of poly-1-lactic acid have been reported at 3.2%26 and granulomatous reactions up to 12%27; however, biopsy-confirmed granulomatous reactions were reported by Vleegar at 1.1%.26 No immunologic panels were performed on any of the proven granulomatous reactions.28

DISCUSSION

Soft tissue filler injections are the second most common nonsurgical treatment in the United States after botulinum toxins. The three most commonly injected filler materials are HA, calcium hydroxylapatite, and poly-lactic acid. Their efficacy and safety have been well established, resulting in their approval for cosmetic use by the U.S. Food and Drug Administration; however, they are non-autogenous, and, as with all implanted materials, foreign body reaction may occur.

The stimulating fillers, poly-lactic acid and calcium hydroxylapatite, create an inflammatory response and together with their collagen-stimulating properties contribute to their effectiveness as soft tissue fillers. These limited inflammatory reactions resolve when the product is degraded.29 HA hydrogels, which are highly hydrophilic and exchange water freely with the host, may also invoke mild foreign body reactions, but to a lesser extent.30 Hydrogels are also known to stimulate collagen formation and displace tissue, but their filling properties are likely complemented by their hydrophilic properties, which attract water to the tissue site.

Complications to soft tissue fillers that include biopsy-proven granulomatous reactions often are reported as hypersensitivity reactions. This diagnosis suggests an immune-mediated or allergic phenomenon; however, a classic type I hypersensitivity response should include antibody formation and predispose the patient to an equal or greater immunologic response after a second exposure to the sensitizing material. Reports supporting this mechanism are sparse. Foreign body reactions to viscoelastic agents implanted into the body are not uncommon; however, they are not the same as granulomas, which are robust, type IV, T cell-mediated delayed hypersensitivity reactions consisting of an inflammatory infiltrate composed of histiocytes and epithelioid and giant cells. Foreign body reactions differ from granuloma reactions mainly by the proportion and arrangement of lymphocytes, plasma cells, neutrophils, eosinophils, and multinucleated giant cells, and also by the amount of polymorphous exudates and sometimes the presence of necrosis.29 The time between injection and the first appearance of a foreign body granuloma is usually 6 to 24 months.29

The symptoms and clinical course of most reported hypersensitivity and granulomatous reactions after filler treatments appear inconsistent with the classic type I or type IV hypersensitivity reaction and are more consistent with an infectious cause. It has been shown that hydrogel fillers contaminated with bacteria can cause a foreign body response mimicking an allergic reaction.10,31 Many instances of delayed hypersensitivity reactions have been reported.1,6,9,24,52–55 The onset of effects ranged from 5 days1 to 2 months,22 most occurred between 1 and 6 weeks.6,9,16,24,33,54,56 Most cases consisted of painful erythematous nodules,1,6,9,24,33,56 some of which progressed to fluid-containing abscesses of varying severity6,32,56 that contained purulent6,52 or nonpurulent1 fluid.

Most patients were treated with oral antibiotics1,6,24,33,43 although bacterial cultures were consistently negative.1,6,32–34 The results of one intradermal rechallenge was also negative.24 Other treatment consisted of intramuscular34 and intralesional triamcinolone acetonide injections6,9,16,24,53 and oral prednisone or methylprednisolone.5,9,16,24,35 In some instances, surgical incision and drainage was required.1,24,32,36 Some cases followed a prolonged, remitting course.1,9,33,34,36 A foreign body granuloma was noted on biopsy in several cases after a protracted course.1,33,36

One report included a patient known to the senior author who was treated with intralesional steroids and
had a complicated course that finally resolved after a prolonged course of clarithromycin. The author subsequently treated other unreported “hypersensitivity reactions” after intradermal treatment with HA, calcium hydroxylapatite, and poly-L-lactic acid that have resolved after treating with antibiotics and hyaluronidase (HA and calcium hydroxylapatite reactions). Other authors have suggested an infectious entity was responsible for a reported hypersensitivity reaction despite a pathology evaluation revealing a histiocytic infiltrate. A similar clinical course for the majority of reported “hypersensitivity reactions” after treatment with nonpermanent fillers since 2000 are more consistent with bacterial infections and are similar to well-described biofilm reactions associated with permanent fillers. Biofilms can present as sterile abscesses or chronic indolent inflammation and infections that are up to 1000 times more resistant to antibiotics. They do this by reducing their metabolism, cell-to-cell signaling, and by secreting a protective extracellular HA and polymorphic glycosylated matrix, which prevents white blood cell penetration.

The nature of the protective matrix may explain the reason why use of hyaluronic acid has been successful for the treatment of reactions caused by non-HA soft tissue fillers. Treatment of Streptococcus intermedius biofilms with hyaluronidase has shown a reduction in biofilm mass by 66%. Patients with chronic biofilm reactions often present with a localized area of persistent inflammation, sterile abscess, and discomfort with an elusive cause that is often mislabeled as a hypersensitivity reaction. Oral and/or intraleSIONAL steroids and nonsteroidal anti-inflammatory drugs (NSAIDs) are routinely used as first-line agents to treat hyperinflammatory conditions such as type I and type IV hypersensitivity reactions; however, the treatment of inflammation secondary to an infectious biofilm with steroids and NSAIDs has been associated with a worse outcome and prolongation of the infectious biofilm. The steroids and NSAIDs mask the contamination by providing a brief reduction in inflammation; however, treatment with steroids favors bacterial assembly into a biofilm and thereby increases bacterial resistance, and it remains ready to resurface after steroids and NSAIDs are discontinued.

Biofilms have been associated with bacterial contamination of soft tissue fillers during the implantation procedure or after the introduction of a contaminating agent into an already implanted product. Biofilm-related reactions appear more likely to occur within the first 2 weeks after the filler implantation. This suggests the need for added precautions if dental infections or facial trauma occurs within 2 weeks of a filler placement. Other factors leading to infectious complications and biofilm exposure include high-volume bolus filler injections.

**MANAGEMENT OF COMPLICATIONS**

Successful treatments of early infectious complications from biofilm reactions have included high-dose administration of a prolonged course of broad-spectrum antibiotics such as fluoroquinolone (ciprofloxacin, levofloxacin) or macrolide (azithromycin, clarithromycin) antibiotics. Laser lysis and surgery have been suggested as treatments for persistent and resistant infectious complications secondary to biofilms. Sterile technique together with prophylactic measures prior to or immediately after treatment with permanent fillers can reduce the risk for infectious complication. An injection technique that minimizes the number of injection sites and avoids bolus injections, injection through previously placed filler, and injection through oral or nasal mucosa is recommended to minimize the incidence of complications secondary to bacterial contamination. A benzalkonium chloride wash is recommended prior to injection and has demonstrated superior antiseptic efficacy to that of alcohol.

An algorithmic approach to treating filler complications has been successfully used by the senior author (Fig. 1). Chronic inflammation of greater than 2 weeks

![Figure 1](image_url)  
**Figure 1** Inflammatory reactions secondary to a filler have been successfully treated using an algorithmic approach that uses hyaluronidase and antibiotics, avoiding the use of steroids.
or resistant inflammatory reactions secondary to a filler have been treated by the senior author with hyalurondiase (10 to 30 units mixed 1:1 with saline), regardless of the filler used. In addition, patients have been treated with antibiotics (ciprofloxacin or clarithromycin) for up to 6 weeks (Fig. 2). It is important to avoid all forms of steroids and NSAIDs as their use may encourage bacterial assembly into a biofilm.40

Biofilms have also been shown to be susceptible to 5-fluorouracil (5-FU) through inhibition of DNA synthesis and reduction of the biological activity of RNA in both Gram-positive and Gram-negative bacteria.46,47 Should the complication persist or if steroids have already been used, it is recommended that the indurated area be treated with an intralesional 5-FU (15 to 40 mg) repeatedly every 4 weeks. The most resistant reaction treated by the senior author required a series of three injections over 3 months prior to resolving.

If the induration remains persistent despite repeated treatments with 5-FU, consider laser lysis, incision and washing out of the cavity with antibiotics (lincomycin) or, at last resort, surgical excision.

CONCLUSION
The possibility of a bacterial contaminant should be considered in any acute or chronic inflammatory reaction regardless of the product used although there have been no confirmed reports of biofilm reactions with the nonpermanent fillers currently used in the United States. Hypersensitivity reactions after filler treatments are likely to be secondary to bacterial contamination. Steroids and NSAIDs seem to aggravate and prolong the inflammation, and early treatment with antibiotics is recommended.

REFERENCES
13. Narins RS, Dayan SH, Brandt FS, Baldwin EK. Persistence and improvement of nasolabial fold correction with nonanimal–stabilized hyaluronic acid 100,000 gel particles/mL filler on two retreatment schedules: results up to 18 months on two retreatment schedules. Dermatol Surg 2008;34(Suppl 1):S2–S8; discussion S8
19. Augus JE, Affleck AG, Leach IH, Millard IG. Two cases of delayed granulomatous reactions to the cosmetic filler Dermalive, a hyaluronic acid and acrylic hydrogel. Br J Dermatol 2006;155:1077–1078
41. Pecharol D, Petersen FC, Schie B. Role of hyaluronidase in Streptococcus intermedius biofilm. Microbiology 2008;154(Pt 3):932–938