Is a Skin Test Necessary Prior to Bovine Collagen Gel Suspended Polymethylmethacrylate Microsphere Aesthetic Injections?

Abstract:
Background: A key to facial rejuvenation is treatment of facial wrinkles and volume deficiencies created by the aging process.1 Dermal fillers are the second most common minimally invasive aesthetic procedure performed in the United States.2 There is a long-term dermal filler option of bovine collagen gel suspended polymethylmethacrylate, known as Bellafill (ART).

Objective: Is a skin test necessary before bovine collagen gel suspended polymethylmethacrylate injection?

Methods & Materials: A retrospective review from 2009 to 2013 and a prospective evaluation of patients from 2013 to 2016 in ART patients was undertaken. Patients were queried about lidocaine or red meat allergy. Patients confirming neither allergy received ART. The study evaluated a comprehensive facial volumetric rejuvenation protocol, including a combination of ART and a hyaluronic acid filler.4 Chart review and injector survey data were tabulated.

Results: The total number of patients treated from 2009 through 2015 was 1,513. A total of 2,875 ART syringes were injected. There were no injector observed allergic reactions to any ART aesthetic treatment.

Conclusions: ART is a valuable component of the comprehensive facial rejuvenation protocol. When patients confirm a lack of allergic response to lidocaine and red meat, ART can be injected safely without performing a skin test.
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Introduction

A key to facial rejuvenation is treatment of facial wrinkles and facial volume deficiencies created by the aging process.\textsuperscript{1} Dermal fillers are recognized as the most common treatment alternative to improve facial volume loss and the second most common noninvasive or minimally invasive procedure performed (behind neuromodulators) in the United States.\textsuperscript{2} There are temporary dermal fillers (less than two-year duration) and one long-term dermal filler that is approved for use throughout the United States by the Food & Drug Administration (FDA). The long-term filler is bovine collagen gel suspended polymethylmethacrylate microspheres, known as Bellafill\textsuperscript{®} or Artefill (ART), which is manufactured by Suneva Medical, Inc, San Diego, CA.\textsuperscript{3} Although the FDA approved liquid silicone injections, some states forbid the use of this permanent filler material, including the State of Nevada where I practice.

When a patient elects a permanent treatment modality for facial volume replacement, the following options are presented: 1. a silastic facial implant 2. facial fat grafting or and 3. suspended polymethylmethacrylate (PMMA) microspheres in a bovine collagen gel. A comprehensive facial volumetric rejuvenation protocol was presented to include the combination of ART with a hyaluronic acid dermal filler.\textsuperscript{4} The hyaluronic acid filler used in the comprehensive facial volumetric rejuvenation protocol was Restylane Lyft (PER\textsubscript{1}) ART was FDA approved in 2006 for the correction of facial wrinkles, known as nasolabial folds, by volume replacement (Figure 1).\textsuperscript{5} This dermal filler was sold in the United States as Artefill and manufactured by Artes Medical. Suneva Medical, Inc., bought the rights to this dermal filler in 2009. The formulation of ART by Suneva
Medical, Inc. is identical to that of the previous manufacturer. In 2014, there was a name change in the United States to the name used in Canada, Bellafill.

The filler became the only FDA approved dermal filler for the correction of moderate to severe, atrophic, distensible facial acne scar on the cheek in patients over the age of 21 years in 2014. It has been proven to be safe and effective for correction of nasolabial folds through 5 years in the longest and largest prospective dermal filler study ever compiled. Although these indications are noted in the manufacturers IFU, in clinical practice, ART is used anywhere in the face that volume replacement is desired, except for the bulk of the upper and lower lips (Figure 1). In the 5-year post-approval study, no treatment-related serious adverse events or unanticipated adverse events were noted. The general adverse event profile was similar to prior nasolabial fold (NLF) studies. The PMMA microspheres create a matrix that supports and stimulates endogenous human collagen synthesis from fibroblasts with neovascularization. As the bovine collagen completely absorbs within 2 weeks to 3 months, the host collagen yields persistent residence in the facial soft tissues (Figure 2). Across the four ART studies, the granuloma rate was noted to be 1.2%. In the 5-year post-approval study, granulomas occurred infrequently (1.7%). The majority of granulomas were classified as mild to moderate in severity and the ongoing granuloma rate at the end of the study was <1%. The initial manufacturer of ART, Artes Medical, Inc., submitted to the FDA the need to perform skin testing prior to the aesthetic filler injection. They were using the information from the FDA submission of Zyplast™ and Zyderm™ and the information from the European use of Artecoll.
The FDA states that the manufacturer must instruct U.S. physicians that a skin test should be performed prior to ART dermal filler injections. According to the Instructions for Use brochure, the skin test contains only lidocaine and bovine collagen. The microspheres of PMMA are an inert substance that does not require skin testing.

The Zyderm™ IFU available at the time of Bellafill’s FDA submission states: Sensitization reactions to injectable collagen implants have occurred in 1-2% of treated patients. Most reactions have been of a hypersensitivity nature and have consisted of erythema, swelling, induration and/or urticaria at implantation sites. Often these reactions have occurred following an unrecognized or unreported positive collagen skin test. Most of the remaining responses occurred in patients who became sensitized to Zyderm™ collagen implant at some point during their course of treatment. Typically, these allergic reactions may be intermittent or continuous in nature and persist between one and nine months (mean four months). Approximately 80% of these reactions occur within four weeks following the sensitizing dose.

The recommendations are to undergo the skin test 28 days prior to the aesthetic ART injection. This timing was related to the submission request from Artes Medical, Inc. during the FDA approval process. The 28-day skin test timing was identical to the submission to the European Union of Artecoll, which was due to the wait period from bovine collagen fillers, known as Zyplast™ and Zyderm™. If there is a positive skin reaction to ART (Figure 3), then the ART dermal filler injection is not recommended.

Health Canada is the governing organization for the use of medications in Canada. Health Canada does not require the manufacturer to instruct physicians that a skin test is necessary prior to ART injections. In Canada, a skin test prior to treatment is optional.
The American Academy of Allergy, Asthma & Immunology practice parameters and guidelines, last updated in 2008, states that skin testing for diagnosis of local anesthetic allergy is limited by false-positive reactions.\textsuperscript{10} The practice parameters recommend a provocative challenge as the gold standard. Nearly all the local anesthetic adverse reactions are due to vasovagal reactions, psychosomatic, anxiety, or toxic effects (most commonly excess dosing during liposuction surgery).\textsuperscript{10}

IgE-mediated or anaphylactoid reactions to local anesthetics are extremely rare and much more common in ester than amide local anesthetics. The allergy reaction to amide local anesthetics are so rare that there only a few case reports.\textsuperscript{11,12} In patients who tolerate a provocative challenge without an adverse reaction, there is a false-positive skin test in 19\% of those with a negative history of an allergic response and 9\% in history-positive patients.\textsuperscript{13} In summary, because of the low pretest probability of IgE-mediated local anesthetic allergy and the incidence of false-positive results, it is unclear whether intracutaneous tests have any benefit in the diagnostic approach to local anesthetic allergy.\textsuperscript{14,15}

Rare patients may also have a positive skin test result to the non-formaldehyde preservatives methylparaben or thimerosal added to local anesthetics.\textsuperscript{16} Some of these tests are also false-positive, because subcutaneous challenges to local anesthetics with methylparabens are often negative.\textsuperscript{16} Alternatively, in patients with a history of adverse local anesthetic reactions, subcutaneous local anesthetic challenges using a graded incremental approach has been reported to be safe (n=236).\textsuperscript{14} Re-challenge without a prior skin test was reported to be an easy and cost-effective alternative to skin testing.\textsuperscript{17}

The other component in the manufacturer supplied skin test is an animal protein, bovine collagen. Most of the concern for an allergic reaction is due to the bovine collagen
present in ART. Bovine collagen type I has been found to be an allergen in beef. The IgE reactivity is to the alpha 1 or the alpha 2 chains of the bovine type I collagen molecule. Serum samples were taken in children (n=10) with anaphylaxis to vaccines formulated from gelatin with a bovine collagen base. The IgE antibodies in their serum reacted to alpha 2, but not alpha 1 collagen bovine chains. Similarly, the mast cells sensitized with the pooled sera in the children showed alpha 2 chain specific histamine release, but not to the alpha 1 chain. Unfortunately, there is no IgE anti-bovine collagen in vitro test currently available. A scientific study reviewing the literature and presenting a case study revealed that clinical reactions to collagen are rare. However, they concluded that patients with a history of allergic reactions to bovine collagen-derived products should be investigated.

A European clinical trial of 1,280 patients revealed only one patient with a systemic allergic reaction reported to the manufacturer of the same collagen used in Artecoll and ART. Lemperle et al. observed only two acute allergic reactions among more than 3,000 patients after Artecoll implantation. Interestingly, both patients had negative skin testing prior to treatment. The author stated that the allergic reaction cannot be prevented by double testing.

Four ART published studies used for FDA approval (Table I) noted two exclusion criteria questions, which were queried based on the label contraindications listed below:

1. Do you have a known hypersensitivity or previous allergic reaction to any of the components of the study device (including lidocaine or any amide-based anesthetic), or have a history of allergies to any bovine collagen products, including but not limited to injectable collagen, collagen implants, hemostatic sponges, and collagen-based sutures.
2. Are you undergoing or planning to undergo desensitization injections to meat products. 

A total of 1,794 patients have been treated with ART across four U.S. clinical studies (Table I). There were 17 (1.06%) patients in these four ART published studies that had admitted to having one of the exclusion criteria to the dermal filler injection. 

The instructions for use (IFU) pamphlet contraindication section states:

“Bellafill contains bovine collagen and is contraindicated for patients with a history of allergies to any bovine collagen products, including but not limited to injectable collagen, collagen implants, hemostatic sponges, and collagen-based sutures, because these patients are likely to have hypersensitivity to the bovine collagen in Bellafill. Bellafill is contraindicated for patients undergoing or planning to undergo desensitization injections to meat products, as these injections can contain bovine collagen.”

“The dermal filler of suspended polymethylmethacrylate microspheres contains the following components: bovine collagen 3.5%, phosphate buffer 2.7%, sodium chloride 0.9%, lidocaine hydrochloride 0.3% and water 92.6%.” The syringes are presently hand filled from a solution combining 6 cc (23%) of PMMA with 20 cc (77%) of bovine collagen gel. Per FDA requirements, the collagen gel is acquired from one herd of cows located outside Philadelphia, PA, USA. Once manufactured in a GMP laboratory in San Diego, California, it is transported in FDA approved Styrofoam insulated transport boxes to the practice location. The manufacturer recommends refrigerator storage immediately upon arrival at standard domestic refrigerator temperatures.

Material and Methods
Patients presenting for facial rejuvenation first completed a detailed history to assess their overall health to deem them safe to undergo an aesthetic procedure. A retrospective review from 2009 to September 2013 and a prospective evaluation of patients from September 2013 to May 2016 of those patients that elected ART was undertaken. For the first six months of the practice using ART, skin testing was performed. Thereafter, patients were asked if they had a local anesthetic or lidocaine allergy. Additionally, they were asked if they consumed red meat and if they ever had any adverse reaction. If patients confirmed neither an allergy to lidocaine or red meat, then ART was administered without a skin test.

When the decision to proceed with an aesthetic treatment is confirmed, the predicted number of syringes to be used is removed from the refrigerator, patient photographs area performed, the consent form is reviewed with the patient, the patient signs the consent form after all questions are answered.

Chart review and a survey of the observations of the three treating registered nurses as well as those of the medical director were tabulated. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Results:
The total number of patients treated from 2009 through 2015 was 1,513, composed of 83% woman and 17% men with a mean of 51 years of age, SD ± 14.7 (Table II). The total number of syringes injected was 2,875. Throughout the treatment time period from 2009 until the end of 2015, there was a mean of 1.9 number of syringes per treatment, with 2.9 months between treatment sessions with the total number of syringes used per
patient as follows: 0-5 44%, 6-10 33%, 11-15 11%, ≥16 11%. There was an increase use of ART over time compared to HLA fillers with 801 syringes injected in 2015 alone. No registered nurse in the practice observed any skin reactions at the site of the skin tests supplied by Suneva Medical, Inc. in any patient. There were no observed allergic reactions to any patients injected with ART. There were only three patients during the treatment period since 2007 that had any type of reaction at the injection sites (Table III). These three patients had a firm area that developed greater than four months after the injection. This reaction was assumed to be a granuloma, although no biopsy was performed for confirmation. All of these firm areas resolved to patient satisfaction with one to three triamcinolone injections.

Discussion:
Facial volume replacement is paramount to rejuvenation. When a long-term or permanent alternative is sought, a discussion of silastic implants, autologous fat transfer and ART filler should be reviewed. When the ART filler is chosen, there is an important question to be answered prior to treatment: Is a skin test mandatory?

The FDA currently states to the United States manufacturer, Suneva Medical, Inc., that it must instruct physicians that a skin test should be performed prior to the ART dermal filler injection. The skin test supplied by Suneva Medical, Inc. contains only lidocaine and bovine collagen. There is no PMMA in the skin test. The scientific allergy and immunology literature confirms that the risk of an amide local anesthetic reaction is exceedingly rare. Additionally, this literature confirms that skin testing for a local anesthetic allergic response is unpredictable. Clinical history of previous reaction is the most accurate diagnostic tool. From the authors experience,
most of the allergic responses to local anesthetic agents is due to the added preservatives, such as methyparaben, or stabilizers, such as metabisulfite.

The allergic response to bovine collagen is also rare. Skin testing alone for a bovine collagen allergy has a low sensitivity and specificity. ART has a different chemical structure than previous collagen aesthetic injection products, such as Cosmoplast™, Cosmoderm™, Zyplast™, and Zyderm™. ART’s collagen gel is partly denatured. Denatured collagen protein may have less antigenicity. The collagen gel is also not cross-linked and there is some evidence that non-crosslinked collagen may have less antigenicity.

The processing of the collagen of ART is consistent with the proteolytic enzyme method used to remove telopeptides. There may be a potential immunologic benefit of using collagen that has telopeptides removed. There is no direct clinical evidence and there is paucity of data confirming the immunoreactivity of specific epitopes.

The incidence of allergic reactions to ART is rare with no reported instances in this current study with 2,875 syringes injected in 1,513 patients. And combining three studies with the highest number of treated patients (which includes this data) revealed three allergic reactions in 5,793 patients or 0.05%. Two of the three reactions had a negative skin test prior to aesthetic ART injection.

Conclusions:

The data from the current study suggests that if patients deny a history of an allergic reaction to both lidocaine and red meat, then ART can be injected safely without performing a skin test. The literature supports the low allergic response rate of both an amide local anesthetic and the type of processed bovine collagen in ART by the human
host. Suneva Medical, Inc. is currently submitting data to the FDA to change the requirement for skin testing notifications to physicians prior to injecting the aesthetic treatment dosing.

Conflict of Interest:
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References:


16. Macy EM, Schatz M. Immediate hypersensitivity to methylparaben causing false-positive results of local anesthetic skin testing or pro-active dose testing. Permanente J 2001;6:17–21.


Table 1:

Skin Test Data in the Bellafill* Trials

<table>
<thead>
<tr>
<th>Study Description</th>
<th># positive patients / total number of patients in study (total n=1,794)</th>
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<tbody>
<tr>
<td>Artefill Open-Label Study §</td>
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<tr>
<td>Artefill Pivotal Study</td>
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</tr>
<tr>
<td>Artefill 5-Year Post Approval Study</td>
<td>8/1211</td>
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<tr>
<td>ArteFill for Acne Scar</td>
<td>3/175</td>
</tr>
</tbody>
</table>

*Artefill rebranded to Bellafill in the U.S. 2015
§ Data from initial FDA submission, contained in IFU³

Table 2. Patient Population

- Patients: 1,513
- Female: 83%
- Male: 17%
- Mean Age: 51.0
- Standard Deviation: 14.7

Table 3. Patient Clinical Data

- Patients: 1,513
- Syringes Injected: 2,875
- Allergic Reactions: 0
- Suspected Granuloma Reaction: 3
- Granuloma Resolution: 100%

Figure 1

38 yo woman requesting facial volume replacement using Bellafill (6 syringes) to tear trough, nasojugal groove, & submalar areas. Juvederm HC to upper and lower lips. A. B. C. Before D. E. F. After 6 months
Figure 2

A. PMMA-Collagen gel immediate post-injection.
B. PMMA-Collagen gel four weeks after injection.
(Courtesy of Suneva Medical, Inc, 2016). Reveals human collagen formation (off-white lines) produced by fibroblasts (blue cells) around each microsphere (large grey spheres) with microvascularization formation (red vessels). The smaller the microspheres, the larger their combined total surface area and the increased surface area promotes a greater amount of collagen deposition.

Figure 3

Positive erythematous skin reaction to upper extremity Bellafill skin testing (Courtesy of Suneva Medical, Inc, 2016). A positive response consists of erythema of any degree, induration, tenderness, and swelling, with or without pruritus.
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1004x1507mm (72 x 72 DPI)
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