The Biology of Facial Fillers

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ABSTRACT

The biologic behavior of a facial filler determines its advantages and disadvantages. The purpose of this article is to look at the relevant biology as part of a logical basis for making treatment decisions. Historical perspectives and biologic characteristics such as local tissue reaction (including phagocytosis and granulomatous inflammation) cross-linking, particle concentration, immunogenicity, biofilm formation, gel hardness, and collagen neogenesis are considered. Bovine collagen is the most immunogenic facial filler. Porcine and bioengineered human collagen implants have very low immunogenicity, but allergic reactions and elevations of IgG are possible. Cross-linking and concentration affect the longevity of collagen and hyaluronic acid fillers. Gel hardness affects how a hyaluronic acid filler flows through the syringe and needle. Calcium hydroxylapatite, poly-L-lactic acid, and polymethylmethacrylate fillers have been shown to stimulate collagen neogenesis. It appears that any facial filler can form a granuloma. Bacterial biofilms may play a role in the activation of quiescent granulomas. Various authors interpret the definition and significance of a granuloma differently.

KEYWORDS: Facial filler, phagocytosis, granuloma, cross-linking, immunogenicity, biofilm, collagen neogenesis

There are numerous review articles that discuss the specific properties and behaviors of the various facial fillers.[1–3] The biologic properties of the fillers are usually discussed in these articles to explain local tissue reactions, longevity, and potential for short- and long-term complications. It is the biologic behavior a facial filler that determines its particular advantages and disadvantages. Other articles in this monograph will delve more deeply into outcomes and techniques for each filler. The purpose of this article is to look at the relevant biology as part of a logical basis for making treatment decisions.

First, we will discuss the science of what seem to be the predominant biologic characteristics of facial fillers. Next, we will discuss how those principles influence the behavior of the individual fillers. We will confine the discussion primarily to U.S. Food and Drug Administration (FDA) approved facial fillers: collagen, hyaluronic acid, calcium hydroxylapatite, poly-L-lactic acid, and polymethylmethacrylate microspheres in bovine collagen. Because of its historical significance and the early lessons learned for facial fillers, however, we will also discuss silicone.

Arguably, the most important biologic characteristic of any facial filler is the nature of the local tissue reaction around the implant. Inflammation around the implant particle is normal and always occurs.[3] The ideal facial filler should cause as little immediate or late adverse tissue reaction as possible. A primary object of the science is to control this inflammation so that it does not significantly decrease the longevity of the product or lead to chronic or recurrent granulomatous reactions that can become troublesome to the patient. Predictable and safe longevity is, after all, a very important determinant of patient satisfaction. Graivier and others have noted that any facial filler can lead to a granuloma.[3] An active granuloma[4,5] that causes pain or visible inflammation in

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the skin is certainly troublesome. On the other hand, some fillers seem to be able to stimulate a controlled amount of inflammation in the tissue that has a positive effect on the efficacy and longevity of the product.[3]

Physical properties such as particle size, shape, cross-linking, and particle concentration can influence the biologic behavior of the filler. The biologic composition of the filler also determines whether or not it can be stored at room temperature and may be a factor in the risk of biofilm formation around the particles.

Phagocytosis is the body's normal local tissue reaction to a foreign body, living or inert (e.g., bacteria, suture material, splinters, and facial fillers). It is probably the single most important factor in determining the longevity of a filler.[6] Phagocytosis, however, is still not completely understood.[7–9] The degree to which phagocytosis is successful and the amount of time it takes varies widely.[6] Circulating granulocytes, the polymorphonuclear leukocytes (neutrophils, basophils, and eosinophils), can also move into the soft tissue and are the first line of defense, especially the neutrophils and eosinophils. Agranulocytes, the mononuclear leukocytes that include monocytes, lymphocytes, and macrophages, are next. Macrophages are transformed monocytes that have left the circulation and settled in the extravascular tissue.[10] There is also a population of transformable monocytes that resides in the soft tissues.[10] Neutrophils and macrophages are the primary scavengers.[4,5,10] If polymorphonuclear cells are unable to successfully phagocytose the particle, macrophages become more dominant in the process. Macrophages generally attack larger foreign bodies. Neutrophils have a life span of a few hours in blood and a few days in the soft tissue. Monocytes circulate for 8 to 70 hours and slowly migrate to the surrounding tissues. After 8 to 12 hours, they differentiate into macrophages. Macrophages typically have a life span of several months and replace themselves at a rate of ~1% per day.[10]

There are three phases of phagocytosis[4,5]:

1. *Recognition and Attachment.* Attacking leukocytes (macrophages for larger particles) release opsonins such as the C3bi component of complement and the Fe portion of IgG that facilitate the attachment and trigger phase 2.
2. *Engulfment.* Facilitated by opsonins, pseudopods from the leukocyte extend around the particle, ultimately forming an intracellular vacuole.
3. *Killing and degradation.* There is a release of lysozymes, other enzymes like collagenase and hyaluronidase and an oxidative process involving the release of so-called superoxide ions and production of hydrogen peroxide.

Much is still not known about what determines effective phagocytosis. Macrophages can ingest up to ~25% of their volume per hour.[10] *Particle size* is important in phagocytosis, but size is not the sole determinant of effective phagocytosis.[9] There is not a simple correlation between particle size and the efficacy of phagocytosis.[6–9] Particle size seems to primarily influence phagocytosis in cases where the particle volume is greater than the volume of the phagocyte.[8] Phagocytic response seems to decrease with increasing particle size.[7,8,10] Most circulating neutrophils are 8 to 10 μm in diameter; circulating monocytes are 12 to 15 μm; macrophages are 25 to 30 μm.[10] Generally, particles smaller than 15 to 20 μm can be phagocytosed and transported to local lymph nodes, but there are no absolute rules as they relate to particle size.[4–6,11] Murine macrophages (13.8 μm in diameter), for example, can ingest particles up to 20 μm diameter. On the other hand, Ishikawa et al[12] have shown that guinea-pig macrophages take up few 10-μm latex microspheres. Also, individual human macrophages rarely ingest particles greater than 5 μm; particles larger than this generally require aggregated macrophages (i.e., foreign-body giant cells).[10]

Pertinent to our discussion here is the example of calcium hydroxylapatite. The calcium hydroxylapatite (CaHA) filler currently marketed has particles of 20 to 45 μm. CaHA particles less than 15 μm are readily taken up by circulating monocytes that release bone resorptive cytokines. Hydroxylapatite particles greater than 15 μm are not easily phagocytosed, and there is no correlating increase in cytokines.[10]

Surface shape, surface tension, acuteness of contact angles, and surface charge are also important in phagocytosis.[7–11,13,14] Studies performed primarily using spherical particles overlook the role of target shape in phagocytosis.[8] The medical literature on facial fillers talks much about phagocytosis in relation to particle size, but phagocytosis is actually the result of a complex interplay between shape, size, and other factors.[8] Champion and Mitragotri[8] have stated that particle *shape* actually plays a larger role in phagocytosis than does particle size. It seems that in phase 1 of phagocytosis (recognition and attachment), the shape of the particle surface at the point of contact with a phagocyte dictates whether phagocytosis will begin or whether the phagocyte will simply spread over the target and move on. At engulfment, size becomes a factor. It is here that a particle greater than 20 μm, effectively recognized and opsonized, can resist effective phagocytosis. At this point, the size of the particle can prevent completion of engulfment.[8]

The exact effect of *surface charge* is less clear. Whereas some studies have suggested that positive surface charge increases phagocytosis and that negative surface charge decreases phagocytosis, at least one study has shown no significant effect of positive or negative charge on phagocytosis.[10] Whether a particle is hydrophobic or hydrophilic also seems to play a role in

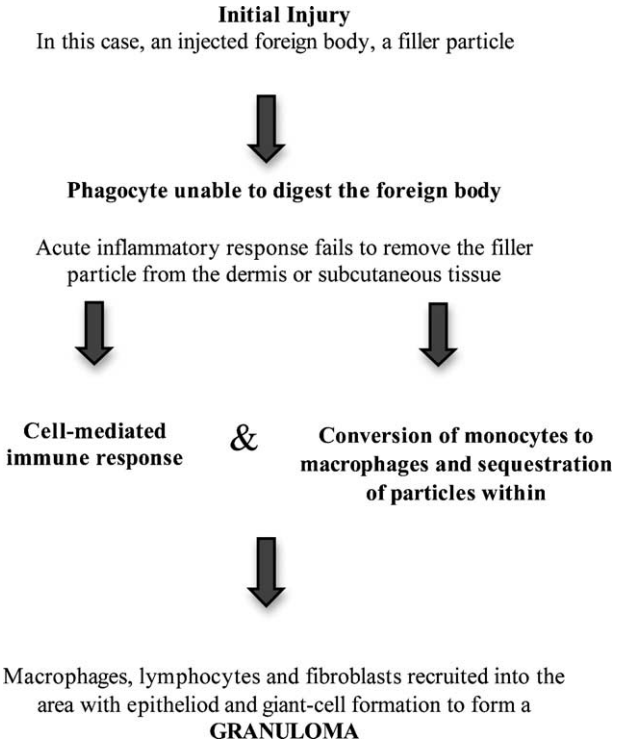

Figure 1 Granulomatous inflammation/granuloma formation. (Information adapted from Rubin E, Farber JL. Pathology. 3rd ed. Philadelphia, PA: Lippincott-Raven; 1999:72. Adapted with permission.)

phagocytosis. *Hydrophilic* particles are less readily phagocytosed.[10] Again in the murine model, particles with hydrophobic surfaces are more readily phagocytosed by peritoneal macrophages.[10] However, particles with more hydrophilic surfaces can become more hydrophobic by absorption of IgG, making them more susceptible to phagocytosis.[10] Hyaluronic acid particles are strongly hydrophilic.

The failure of effective phagocytosis can lead to granuloma formation, encapsulation, and sometimes long-term sequelae. *Granulomatous inflammation* occurs when aggregates of activated macrophages (multinuclear giant cells) are unable to ingest their target[4] (Fig. 1). In these *granulomas*, the macrophages take on a characteristic epithelioid appearance. They begin to look like but do not become squamous cells. The aggregates of these epithelioid cells are surrounded by lymphocytes, which can secrete cytokines responsible for ongoing macrophage activation. Twenty or more macrophages can coalesce into multinuclear giant cells 40 to 50 μm in diameter. These are called foreign-body giant cells when the foreign body can be seen inside the cell on light microscopy and Langerhans giant cells when the nuclei form a horseshoe pattern.[5] In older, more stable granulomas, there develops a surrounding rim of fibroblasts and connective tissue (capsule) that contains the foreign body.[4] The granuloma "walls off" the foreign body, preventing continuous destructive inflammation at the

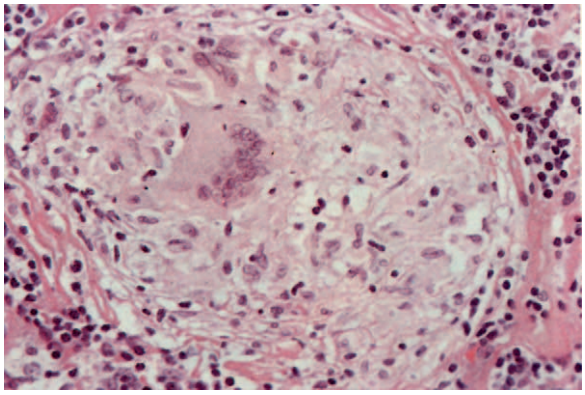

Figure 2 A typical granuloma with multinucleated giant cells, epithelioid cells, a fibrous capsule, and lymphocytic proliferation at the periphery. (From Rubin E, Farber JL. Pathology. 3rd ed. Philadelphia, PA: Lippincott-Raven; 1999:73. Reproduced with permission.)

site of the foreign body[5] (Fig. 2). It appears that particles with *irregular surfaces* are more likely to lead to active granulomas.[6] So-called frustrated macrophages seem to have a memory of their failed attempt to phagocytose and may be a factor in granuloma formation. Protective encapsulation of the granuloma seems to favor smoother particles.[6] If the surface of a particle greater than 15 to 20 μm is smooth, it may be more safely encapsulated. If it is irregular, like silicone or paraffin, it may also be encapsulated but can be induced years later to an active granulomatous inflammation. The reason for activation of the "frustrated macrophages" in these previously quiescent granulomas is unclear but can occur after trauma to the area such as another injection or surgery.

A classic example of the protective effect of a granuloma is the caseating granuloma of tuberculosis, but this is also the mechanism by which microdroplets of silicone, tissue expanders, and breast implants become encapsulated. The capsule that forms and thickens in response to some fillers actually accounts for some or most of the augmentation.[3] Macrophages are generally very long lived, usually for months. When they die, the foreign body is released and can cause a recurrence of active, destructive inflammation months or years later.[5] With more inert particles (e.g., polytetrafluoroethylene [PTFE], silicone), there is generally a slower turnover of the macrophages.[5] Although the reported incidence of late granulomatous inflammation is low in the fillers discussed here, it is still a real possibility.

Cross-linking and increasing the concentration of molecules has an inhibitory effect on phagocytosis in collagen implants and in hyaluronic acid (HA) implants (both discussed in more detail later in this article). In both cases, it is an issue of increasing the size and complexity of the particle presented to the phagocyte. The first FDA-approved molecularly cross-linked implant was Zyplast (Allergan, Inc., Irvine, CA) (bovine collagen) in 1983. In 2003, the FDA approved

CosmoPlast (Allergan, Inc.), a human, genetically engineered cross-linked product. Both are cross-linked with glutaraldehyde. In 2008, the FDA approved Evolence (ColBar Life Science, Ltd., Herzliya, Israel), a porcine collagen cross-linked with D-ribose, a natural occurring sugar.[2] In its natural state, HA is a liquid, and most of the HA molecules are free floating. It can metabolized in 12 to 24 hours. Cross-linking decreases the percent of free-floating HA and makes the product more gel-like. This is believed to prolong its effect.

The *concentration* of collagen and HA products also is believed to affect longevity,[2] but not as much as does cross-linking. More highly concentrated collagen products may increase longevity a month or two, but the products generally only last 3 to 6 months. With HAs, however, the positive effect of increasing concentration may be more important.

Immunogenicity is an issue primarily with collagen implants. In 1987, DeLustro et al[15] demonstrated a lower immunogenicity to bovine collagen implant (Zyderm [Allergan, Inc.]) than to commonly used bovine collagen hemostatic agents when tested in guinea pigs and rabbits. There were significant elevations of immunoglobulins to the hemostatic agents. In 1991, however, Frank[16] demonstrated specific anti-Zyderm antibodies (IgG) in a small number of treated patients and volunteers with and without positive skin tests. In 1993, Cukier et al[17] reported dermatomyositis or polymyositis-like syndromes in 9 of 345,000 patients who received bovine collagen injections between 1980 and 1988. Six of the patients had elevations in serum immunoglobulins. They believed that the incidence of dermatomyositis and polymyositis-like syndromes in this population was significantly above that of the general population.[17] Moscona et al[18] have reported a sarcoid-like reaction at the site of a Zyderm injection 2.5 years later in a patient with negative skin testing. Subsequent retesting on the forearm revealed the same reaction.

An acute inflammatory process involving a quiescent granuloma years later may also have an infectious component. As already mentioned, sometimes this can be precipitated by another injection, a surgical procedure, or trauma. Often, however, there is no precipitating event that can be documented. The exact nature of any infectious component of this process may involve a bacterial biofilm.[19,20] *Biofilms* are structured colonies of microorganisms encapsulated in an extracellular matrix that can surround a foreign body. This matrix (also containing nutrients and bacterial waste products) can maintain and protect the microorganisms for long periods of time.[19] Nearly every species of bacteria can form biofilms. Biofilms grow in varied environments, from the hot pools of Yellowstone to the glaciers of Antarctica. Dental plaque is a biofilm, and biofilms can be responsible for chronic urinary tract infections,[21] chronic urinary catheter infections,[21] chronic middle ear infections,[22] and middle ear ventilating tube infections.[23]

Christensen et al[19,20] have postulated that biofilms maintained within the polymer gels of some facial fillers can lead to a low-grade, chronic infection. They studied 33 biopsies from areas implanted with a variety of permanent, relatively nontoxic and nonimmunogenic filler gels submitted by several different surgeons. The samples included a polyacrylamide hydrogel (Aquamid [Ferrsoan, Soeburg, Denmark]), an HA polyhydroxyethylmethacrylate/ethylmethacrylate gel (Dermalive [Dermatech SAR, Paris, France]), and polylactic acid in mannitol/carbomethoxycellulose (New-Fill [Sanofi-Aventis, Paris, France]/Sculptra [Sanofi-Aventis, Bridgewater, NJ]). The inflammatory nodules from tissues injected with Aquamid showed an increased foreign-body reaction and a bacterial "infection." Moderate foreign-body reactions, fibrosis, and some bacterial "infections" were also found in the inflammatory nodules submitted from tissues injected with Dermalive and New-Fill. They postulated that inflammatory nodules can be caused by low-grade infections arising from biofilms.[19,20]

The *hardness* of a gel, measured by the elasticity factor G', and the resulting *viscosity* of the material play important roles in determining flow characteristics in the syringe and tissue.[24] At least in the case of HA fillers, increasing the degree of cross-linking hardens the gel and also increases its initial viscosity and needed overall extrusion force.[24] These relationships are discussed in more detail in the discussion of HA fillers later in this article.

The ability to stimulate *collagen neogenesis* is a characteristic shared by at least three facial fillers, calcium hydroxylapatite, poly-L-lactic acid, and polymethylmethacrylate in bovine collagen. There is one article in the literature that suggests the cross-linked HA may also share this quality.[25]

Having reviewed the relevant biology in general terms, it is worthwhile to examine how these properties may affect each of the fillers more specifically.

SILICONE

Silicone is not currently FDA approved specifically for cosmetic injection. For historical purposes, however, it is worthwhile to include silicone in our biology discussion. Many physicians still consider silicone a valuable tool in facial augmentation and have demonstrated how it can be used safely.[26–29] There are definitely some lessons to be learned from how it has been used.

Liquid silicone was widely used in Germany, Switzerland, and Japan in the 1940s for body contouring. It became popular in the United States in the 1950s and 1960s.[30,31] Large volumes of Dow Corning 360 were

injected for body contouring. Up to 2 L at a time might be injected, and the material could be contaminated with heavy metals. As many as 40,000 patients may have been injected in the arms, buttocks, breasts, and face, especially in California and Las Vegas.[30,31] In an altered form called the Sakurai formula, olive oil was added to try to induce fibroblast formation, which was thought, mistakenly, to prevent migration. Sakurai[30] used this formula in more than 100,000 patients. These large-volume, contaminated injections often resulted in permanent deformities and often disastrous local and systemic complications such as irregularities in contour, chronic inflammation, migration, extrusion, ulceration, silicone granulomas, pooling of materials, pulmonary embolism, chronic infection, and silicone pneumonitis. Organ failure and death occurred all too often.[30,31] Whether or not silicone can cause an autoimmune disorder is still not absolutely clear.[30,31]

From 1965 to 1971, Dow Corning conducted a study of medically pure Dow Corning 360 involving eight physicians. In 1974, the company applied for approval to market the product and about 2 years later started another study with 26 more investigators selected by the FDA. This study ran through 1981. In 1991, Dow Corning failed to submit sufficient safety and efficacy data. By that time, along with the 1992 controversy surrounding alleged autoimmune reactions to leaking and nonleaking silicone gel breast implants, the approval for injectable silicone was doomed for the time being.[31]

Silicone is approved for intraocular injection to tamponade some extensive retinal detachments.[6] It is usually removed from the eye after a year or so.[31] Medical-grade silicone is a clear, oily, colorless and odorless liquid. It is now supplied as a long-chain polydimethylsiloxane oil.[6] Medical-grade products currently available include Adatosil 5000 (Bausch & Lomb Pharmaceuticals, Madison, NJ) and Silikon (Alcon Laboratories, Fort Worth, TX).[6,31] The viscosity of these products at rest are 5000 and 1000 centistokes, respectively.[6] Water is 100 centistokes. Silicone stores easily at room temperature. It is minimally antigenic, permanent, most likely noncarcinogenic,[31] and does not readily support bacterial growth.[30] Even at viscosities of 5000 centistokes, its flows very easily through a 30-gauge needle.

Starting with medical-grade Dow Corning 200 and then Dow Corning 360, Richard Webster in Boston[26,27] (R.C. Webster, personal communications) and Norman Orentriech in New York[28,29] took a different approach to silicone injection. They postulated they could take advantage of the body's ability to encapsulate small amounts of the product because the molecules could not be effectively phagocytosed. By injecting "microdroplets" of "medically pure" silicone into the subdermis, they postulated they could control granuloma formation and produce minimal local fibrosis that would provide the real augmentation. Dr. Webster had a bottle of medically pure Dow Corning 200 and later Dow Corning 360 that he continued to use throughout the rest of his career (R.C. Webster, personal communications). The material had been provided to him by Dow Corning before the commencement of the 1965 studies. As stated in his 1986 paper,[27] this bottle was also cultured regularly; no bacterial growth was ever noted. Their technique took advantage of the fact that the body would encapsulate small amounts of the product. The local fibrosis and "microencapsulation" is what leads to the actual augmentation. The areas to be injected are underinjected, relying on the local tissue reaction to produce the augmentation. The technique involves often <0.005 to 0.01 cc microdroplets injected with a 30-gauge needle and is the predominant technique used today. In Dr. Webster's hands, treatment sessions typically involved only 0.1 to 0.02 cc total, and treatments were 6 weeks to 3 months apart.[26,27] In 1984, Webster et al[26] reported on 524 patients injected with the microdroplet technique over 20 years with minimal complications.[26] They treated wrinkles and scars in addition to doing some facial augmentation and treating postrhinoplasty depressions. The fibrosis that formed was said to give a very smooth, natural augmentation. The affects were permanent.[26]

Acknowledging the difficulty in getting adequate follow-up over a 20-year period, they could find only four patients with complications, all relatively minor. The complications were minimal erythema, infection, nodularity, and anxiety caused by a media report that led to a negative excisional biopsy. Reporting again in 1986[27] on 235 patients with 2811 treatments in various facial areas (excluding the nose) treated for facial augmentation only, they were able to state definitively that the amount of augmentation observed far exceeded the very small volumes of silicone injected. Again, they also concluded that the microdroplet technique was safe and easy to learn.

The controversy surrounding silicone continues today mostly because it is still off-label and because of the medical and ethical disasters associated with its earlier use in very large quantities. Its cosmetic use is illegal by statute only in Nevada, California, and Colorado. The FDA does not have the authority to regulate the practice of medicine,[31] only drugs and medical devices. Some physicians who inject silicone as a filler believe that the FDA Modernization Act of 1997 reaffirms this and readily permits its off-label use.[3,31]

COLLAGEN

There are currently three sources of FDA-approved injectable collagen: bovine calf skin (Zyderm and Zyplast), human genetically engineered dermal fibroblasts (CosmoDerm [Allergan, Inc.] and CosmoPlast), and porcine gastrocnemius tendons (Evolence). Zyderm I

was approved in 1981, Zyderm II and Zyplast in 1983, CosmoDerm and CosmoPlast in 2003, and Evolence in 2008.

The molecular subunit of collagen is tropocollagen. It is a long, narrow rod with a molecular weight of ~300,000.[32] Each molecule consists of three polypeptide chains wound together in a triple helix, which then coils on itself into a superhelix. The most antigenic portion of the collagen molecule is believed to be the nonhelical telopeptide ends.[32] There are naturally occurring covalent cross-links within and between molecules.[33] Release of collagenase during and subsequent to phagocytosis is responsible for its degradation.[2,33] Cross-linking and increasing the concentration of the product have been the main strategies for decreasing the effectiveness of phagocytosis of collagen injectables. Further increasing this natural cross-linking in the molecule prolongs the longevity of the product.[32,34] In animal studies,[32] there was a typical polymorphonuclear response between 12 and 72 hours that resolved in 5 to 7 days and was followed by an influx of vascular channels and fibroblasts. Over a 6-month period, the implant retained its original random character. There was also no evidence of migration or encapsulaton.[32] Early animal studies showed persistence of product at 22 weeks to 6 months.[35] In early studies, biopsies of injected areas in rodents showed no persistence of inflammation and collagen indistinguishable from surrounding host collagen.[36]

Zyderm I, Zyplast, CosmoDerm I, CosmoPlast, and Evolence are all 3.5% collagen (35 mg/mL). Zyderm II and CosmoPlast II are 6.5% collagen (65 mg/mL). Zyderm I and CosmoPlast I are designed for injection into the superficial dermis. Zyderm II, Zyplast, CosmoDerm II, CosmoPlast, and Evolence are intended to be injected into the mid to deep dermis. These products are all buffered to be approximately pH neutral in saline and contain lidocaine to decrease the pain of injection. The human and bovine products must be kept refrigerated. Zyplast and CosmoPlast are cross-linked with glutaraldehyde. Evolence is cross-linked with D-ribose.[2,37]

Evolence is the newest FDA-approved collagen (2008). Porcine collagen more closely resembles human collagen.[2,37] Because it so closely resembles human collagen and because the N-terminals are removed in the production process, it should have significantly decreased immunogenicity.[2,38] In a study of 519 patients, there were no hypersensitivity reactions.[38] There is not currently a large number of long-term studies available. In one small study comparing Evolence with Zyplast (12 patients), there was better persistence of Evolence at 18 months and no elevation of immunoglobulins during the study.[39] In a larger study comparing melolabial fold augmentation of porcine collagen and Restylane (Medicis Aesthetics, Scottsdale, AZ), there was no difference in the effect at 6 months. One percent of the subjects developed asymptomatic IgG antibodies to the porcine collagen.[40] Narins et al[37] have also reported persistence of augmentation at 12 months.

With Zyderm II and CosmoDerm II, initially it was also believed that increasing the concentration helps decrease the rate of phagocytosis.[2,32] Still, the product usually only lasts 3 to 6 months. The higher concentration also created a product for slightly deeper augmentation, mid to deep dermis instead of for the superficial dermis.

Immunogenicity can be an issue with all collagen implants.[15–18] As discussed already, late local and systemic reactions to bovine collagen and elevations of immunoglobulins have been reported.[15–18] Bovine collagen is significantly immunogenic. The incidence of allergic reaction to skin tests is ~3 to 5%.[2,32,38] During its purification and manufacturing process, cleavage of many of the telopeptides decreases but does not eliminate its immunogenicity.[32,36] To the extent that cross-linking decreases the number of telopeptides, it may also decrease the immunogenicity,[33] but this has not been proved definitively. Patients should be skin tested twice with ~1 month between the first and second tests and receive treatment no sooner than 2 weeks after the second skin test.[2] A small percentage of patients can still react to the product after negative skin tests. The author has personally seen a hypertrophic scar form at the site of an initial skin test that required injection with triamcinolone to help it resolve. Patients with documented autoimmune disease should not be injected, and atopic individuals would be expected to have a higher incidence of allergic reactions.[32] The FDA has not required skin testing for human and porcine collagen, but there is also evidence of immunogenicity with these products. The Inamed Human Collagen Immunogenicity Clinical Study showed, with a 95% upper confidence level, that the chance of experiencing a hypersensitivity reaction to CosmoDerm and CosmoPlast was less than 1.3%.[41] Stolman[42] reported on two cases of reactions to CosmoDerm that persisted 3 weeks and 3 months, respectively. One of the patients had a history of a previous skin hypersensitivity to Zyplast.[41] The author has seen localized swelling and persistent erythema with human collagen in one patient previously treated successfully with Zyderm. The areas improved some with 4 weeks of topical steroid but essentially took 4 months to resolve. The areas were raised and red but not itchy. The literature on porcine collagen is still less extensive, but elevations of IgG have been documented.[40]

HYALURONIC ACID

HA, the most common glycosaminoglycan in human skin, naturally binds water in the skin and provides volume. It stabilizes intercellular connections, producing a viscoelastic network to bind collagen and elastin

fibers.[2] The various HA fillers differ in characteristics such as the type and degree of cross-linking, gel hardness, viscosity, extrusion force, gel consistency, and total HA concentration.[24] HA fillers are easily stored at room temperature.

Because the basic monomeric unit of the HA polymer is the same across all species,[24] HA fillers have naturally low immunogenicity, but delayed hypersensitivity can occur.[43] Rates of hypersensitivity reactions are reported to be about 1:5000.[44] The basic structural difference between animal and non-animal (bacterial-based) HA fillers is the length of the polymer chain, 4000 to 6000 monomeric units per chain for non-animal stabilized products (Restylane, Perlane [Medicis Aesthetics, Scottsdale, AZ], Juvéderm [Allergan, Inc., Irvine, CA], Captique [Genzyme, Ridgefield, NJ], Elevess [Anika Therapeutics, Woburn, MA]) and 10,000 to 15,000 units for animal-based products (Hylaform [INAMED, Santa Barbara, CA]). Their immunogenicity seems to be similar. HA is a very hydrophilic molecule, a factor in resistance to phagocytosis.[20] It forms a very highly water-soluble polymer. HA also has a slight heparin-like effect locally that can lead to more bruising compared with that for collagen fillers.[2] HA is the only filler for which there is a specific "antidote-like" substance. Small amounts of hyaluronidase can be used to decrease overcorrections with any of the HA fillers.[45,46] Sall and Ferard[47] have even proposed using a test with bovine hyaluronidase to predict the potential in situ stability of an HA filler.

Cross-linking is essential to the persistence of HA in the tissues.[24] Naturally occurring HA, free HA, floats free in solution, is not cross-linked, and has a half-life of only 1 to 2 days. HA manufacturers cross-link the molecules to form a viscous gel that is more resistant to degradation. These gels provide a more complex chemical structure and more robust physical barrier to the enzymatic and free radical breakdown in the phagocytes.[24] The cross-linked material is more rigid and more durable than the solution with free-floating HA monomeric polymers. The cross-linking materials currently in use, 1,4-butanediol diglycidal ether (BDDE), di-vinyl sulfone (DVS), and *p*-phenylene bisethyl carbodi-imide (BCDI), react with the hydroxyl sites on the HA chains.[24]

All other factors being equal, it is logical that the HA gels with higher degrees of cross-linking should persist longer.[24] However, the relationship of cross-linking to persistence and other gel properties is not so simple. There is a still undefined threshold for the optimum degree of cross-linking. Too high a degree of cross-linking, for example, could make the complex polymer less hydrophilic. It could also increase the immunogenicity of the product by making it look less and less like naturally occurring HA to the phagocytes.[24]

To further understand the ramifications of cross-linking on the biologic and physical properties of the HA gel, it is important to understand the manufacturing process. In one manufacturing method, the cross-linking process results in a large gel mass that must then be "sized," broken up into smaller equal units, so that it may pass through a syringe. This process is proprietary to each manufacturer and often uses a series of sieves or screens to produce a particular, consistent particle size distinctive to that HA.[24] It is generally assumed that smaller particles will be phagocytosed more readily and will degrade faster than larger particles. Another approach to processing the gel mass that results from cross-linking is to homogenize it instead of passing it through a sieve that breaks it up. This homogenized product is a softer gel with a more varied distribution of particle sizes. What is not clear is whether the size variations of the currently available products have real clinical significance on their individual rates of degradation.[24]

Gel hardness, or stiffness, of a particular HA gel is described with the variable G'. G' is a measure of the elasticity of the gel, the force needed to deform the gel. Gel hardness affects the amount of force needed to initially begin pushing the substance through a syringe and needle. The relationship between force and displacement is linear up to a so-called yield point, where the force needed to displace the fluid levels off. After this point, the viscosity of the material is more important than hardness in determining the force necessary to complete the injection.[24]

Gel hardness (G') is affected by HA concentration, the degree of cross-linking, the amount of non–cross-linked free HA, and the particular manufacturing process.[24] With all other factors held constant, increasing the degree of cross-linking hardens the gel and also increases the viscosity and extrusion force. HA products produced with a sieve have a more uniform particle size distribution and tend to have a higher G' value, viscosity, and overall extrusion force than do the smoother, homogenized gels with a broad range of particle sizes.[24] A homogenized HA gel is softer. It has a lower G' value and flows more easily.[24] Viscosity and the resultant overall summary force needed to extrude the product through a syringe and needle after the yield point is also related to the degree of cross-linking, particle size, and the uniformity of particle distribution.

Non–cross-linked free HA is an excellent lubricant. Large amounts of non–cross-linked HA can be added to products produced with sieves so that these products will flow better. The high degree of non–cross-linked HA, however, can decrease the persistence of the product. Smoother products produced with a homogenization technique require less non–cross-linked HA to achieve good flow characteristics. Theoretically, without large amounts of non–cross-linked free HA for lubrication, they should last longer in the tissues.[24]

On gram of highly cross-linked HA can effectively bind up to 3 L of water.[24] Products with

high concentrations of HA are typically below their equilibrium level of hydration and can still bind large amounts of water upon injection. They will produce more swelling, and the physician should be prepared for this.[24]

Restylane, Perlane, Juvéderm Ultra, and Juvéderm Ultra Plus (Allergan, Inc.) are cross-linked in the same way with BDDE.[47,48] Restylane is a non-animal stabilized HA (NASHA) product produced from a bacterial broth of equine streptococci.[2] Restylane is a particulate gel with 100,000 particles per milliliter and a concentration of 20 mg/mL. It is produced with sieve extrusion and has a particle size of ~259 to 300 μm.[48] It is ~1% cross-linked. Perlane is a larger particle version of the same HA with the same concentration of 20 mg/mL but about only ~8000 to 10,000 particles per milliliter due to its larger particle size (~1000 μm). Its degree of cross-linking is the same as with Restylane. Juvéderm Ultra and Juvéderm Ultra Plus are more highly cross-linked (~6% and 8%, respectively) and more homogenous gels, as a result of the homogenization of their manufacturing process. The Juvéderm Ultra and Juvéderm Ultra Plus concentration is 24 mg/mL. Elevess (FDA approved in 2008) is a highly concentrated and highly cross-linked HA. It is cross-linked with BCDI. The concentration of Elevess is 28 mg/mL. It is also the first HA approved by the FDA that contains lidocaine. Hylaform and Hylaform Plus are animal derived HAs, derived from the head combs of roosters. The concentration of each is 5.5 mg/mL. Hylaform Plus has larger particles. Captique is derived from a bacterial source with a concentration of 5.5 mg/mL. Hylaform, Hylaform Plus, and Captique are less cross-linked than Restylane, Perlane, Juvéderm Ultra, and Juvéderm Ultra Plus.

HA products have longevities in the range of 6 to 12 months depending on the factors discussed here and the location of the implant. Restylane, Hylaform, and Captique can be injected into the mid-dermis. More superficial injection can lead to a bluish discoloration called a Tyndall effect as light strikes the implant. The other products are more safely injected into the deep dermis. Longevity studies for off-label injections in the subdermis and just above the periosteum have not been reported. The author found one article in the literature that showed increased collagen synthesis after cross-linked HA (Restylane) injection.[25] Where might collagen neogenesis occur? The HA is not always injected exactly where the physician thinks it is injected. A recent article[49] looked at the actual level of injection in 16 patients undergoing Mohs micrographic surgery in the area of the melolabial crease. The product, Juvéderm, was injected in skin to be excised for the reconstruction phase of the procedure 1 to 4 weeks prior to surgery with satisfactory results. Despite the intention to inject the deep dermis, all 16 patients also had some product in the subcutis, and one patient had product in the superficial dermis. Further studies to elucidate the potential for neocollagenesis will need to specify the exact level of injection.

CALCIUM HYDROXLYAPATITE

Calcium hydroxylapatite implant (Radiesse [Bioform Medical, San Mateo, CA]; $Ca_{10}[PO_4]_6[OH]_2$) was designed as a subdermal implant meant for injection just under the dermis with the needle barely nicking the reticular dermis or not touching it at all. With a particle size of 20 to 45 μm, size is at least one factor in discouraging effective phagocytosis. Originally know as Radiance FN, each syringe of Radiesse contains calcium hydroxylapatite (30%) suspended a in a methylcellulose carrier gel (70%). The gel is composed of water, glycerin, and sodium carboxymethylcellulose.

Nonporous, ceramic calcium hydroxylapatite and macroporous CaHA (which has a highly organized pore structure with pores varying from 10 to 500 μm) permit osseointegration. The large pores of macroporous CaHA readily permit fibrovascular ingrowth. Nonceramic CaHA cement is used widely in reconstructive surgery to repair bony defects. It is slowly replaced by new bone growth.[50,51] CaHA facial implant (Radiesse) is microporous. The 20- to 45-μm particles have pores of only 2 to 5 μm, too small to promote fibrovascular ingrowth and bone formation. There are no documented cases to date of Radiesse injected subdermally forming bone in the subcutaneous tissues. Whether injecting it along the periosteum may promote longer augmentation and bone formation by somehow stimulating periosteum is speculative. The author knows of no articles that definitively demonstrate this potential.

CaHA is minimally immunogenic and easily stored at room temperature. In an early study, Sadick et al[52] treated 113 patients and reported only 7 (relatively minor) complications: transient ecchymosis, submucosal lip nodules, and inflammatory edema. They demonstrated minimal inflammatory response to the product. Over time, the CaHA particles are broken down to calcium and phosphate ions by the phagocytes, but it is unclear how long these ions actually remain in the tissues. Demonstrable duration of augmentation is believed to be usually in the range of 10 to 14 months, but the author has occasionally seen patients who effectively metabolize the product in 6 to 9 months and others who have photographic evidence of some persistent augmentation at 2 years and even 3 years. Carruthers et al[53] looked at plain films and computed tomography (CT) scans immediately after injection. They found a light hazy appearance on plain films in from 13 to 56% of cases.[53] On CT scan, however, a more definitive white haziness was clearly seen in 96.7 to 100% of the cases. Tzikas[54] showed CT scan persistence of the

product 7 months after injection of the lips. The author knows of no studies that have looked at longer term persistence of the product on CT scan.

It seems that CaHA can also promote new collagen formation in the deep dermis/subdermis. Biopsies taken 6 months after injection showed new type I and type III collagen formation around the CaHA particles. There is evidence of collagen formation around the CaHA particles at 16, 32, and 78 weeks.[2,54,55] One study, however, failed to demonstrate any collage neogenesis.[56]

Though not definitively studied, many physician-injectors believe that persistent ecchymosis after injection is related to the depth of injection, the number of passes with the needle, the location of the injection, and manipulation of the product at the time of injection. Persistent erythema for 3 to 6 weeks after injection is rare and is believed by some physicians to be the result of superficial injection and more related to the gel carrier than to the CaHA. This also has not been definitively studied.

Radiesse has a well-recognized tendency to move easily along and through the orbicularis oris muscle and wind up as a deposit, a nodule, under the submucosa just inside the lip.[52,54] For this reason, the manufacturer recommends against using the product for lip augmentation. These nodules can also form on the vermillion surface. They are generally nontender and asymptomatic except for being palpable and visible. Jansen and Graivier,[57] reporting on 609 subjects in 2006, reported that 42 of 338 patients with lip augmentation developed nodules within 6 to 12 weeks. They were treated with triamcinolone and massage. Nodules that did not resolve were excised and sent for pathologic examination. Histologic examination showed foreign-body giants cells surrounding the CaHA microspheres in a "fibrotic extracellular matrix." The authors did not believe that these were granulomas. Otherwise asymptomatic, the presence of foreign-body giant cells and fibrosis does raise some questions, however. In 2007, Sankar and McGuff[58] reported a granulomatous inflammatory reaction in a 51-year-old woman who presented with several red, inflammatory-appearing nodules along the vermillion border "within weeks" of injection. The lesion appeared first as a lump and then "a painful" blister that resolved. At the first examination she had a nontender yellow nodule. Six weeks later, the nodule was biopsied, and two more asymptomatic nodules were noted in the vermillion border. The biopsy showed a foreign-body giant cell infiltration with evidence of chronic inflammation, fibrosis, and localized chronic sclerosing sialadenitis in the minor salivary glands in the area.[58] They state that this is the first and only granuloma found after injection with CaHA. Even in patients with lipoatrophy, who sometimes require 6 to 10 syringes over a short period of time, the author found no other reports with lesions diagnosed as granulomas. Admittedly, comparing histologic findings in reprinted images is less ideal than reviewing the actual slides, but the author saw no significant difference in the histologic images in both articles. The presenting symptoms, however, were certainly different. The initial nodule in the patient presented by Sankar and McGuff[58] had a more inflammatory history.

POLY-L-LACTIC ACID

Like calcium hydroxylapatite, poly-L-lactic acid (PLA; Sculptra) stimulates collagen neogenesis. It is an entirely synthetic, biodegradable, immunologically inert peptide polymer from the α-hydroxy-acid family and is commonly found in absorbable suture material. PLA stimulates fibroblasts to produce collagen. Unlike other facial fillers, however, this is its only effect. The immediate volumizing affect is temporary. PLA is a dermal stimulating agent designed as a volumizer for facial augmentation, not actually a filler.[2,59] It is intended for subdermal injection. Sculptra is currently FDA approved only for treatment of HIV-associated lipoatrophy, but it is widely used off-label for cosmetic purposes. In freeze-dried form it can be stored up to 2 years at room temperature. It must be reconstituted at least 2 hours before use and remains mostly in suspension. Reconstitution the night before seems to help the product dissolve a bit better. It is usually reconstituted with 3 to 5 mL sterile water. After reconstitution, the suspension should be used within 72 hours.[59] Lidocaine can be added to the diluent, and larger volumes of diluent can be used. Vigorous and consistent massage for 3 to 7 days after injection seems to help distribute the particles evenly.

In addition to the PLA, Sculptra, like Radiesse, contains sodium carboxymethylcellulose. In this case, Sculptra acts to help evenly distribute the PLA particles. It also contains mannitol to enhance the lyophilization process.[59] The particles range in size from 40 to 60 μm. Here, too, it seems that particle size at least plays a roll in discouraging phagocytosis. Activation of collagen neogenesis requires granulomatous inflammation[4,5] that slowly dissipates and seems to generally have positive effects with regard to persistence of the augmentation. The large PLA particles attract macrophages (which coalesce to form foreign-body giant cells), lymphocytes, fibroblasts, mast cells, and vascular ingrowth. The capsule that forms by 1 month around the implant is filled with these cells.[60,61] By 3 months, there is about a 20% decrease in the cellular density and thickness of the capsule but a visible increase in collagen fibers. The mast cell population remains high. By 6 months, the thickness of the capsule drops another 20% and the cell count continues to drop. The capsule by then is almost entirely made up of new collagen.[60,61] By 18 months, there is still persistence of new collagen but no significant signs of ongoing granulomatous inflammation.[60,61]

The most common adverse outcome with PLA is also nodule formation. As with CaHA, in most cases, these are believed to be nodules, not true granulomas. They are believed to be related to technical considerations such as dilution, massage, and injection technique and location.[59] The periorbital area is a common location, and the nodules can be long-lasting. Reporting on 7185 treatments with PLA in 2131 patients, Vleggaar[62] noted 66 (3.2%) patients with nonvisible but palpable nodules <5 mm after facial injection. Of these, 26.9% resolved within 3 months. In this study, they were most common in the oral commissures. He also reported 26 (1.2%) patients with visible subcutaneous nodules. In 7.7% of patients, the visible nodules resolved spontaneously within 2 months. By 3 years, all nodules seemed to resolve. The author has three patients who developed nodules of the infraorbital area within 1 month of treatment. They were treated with subcision, saline injection, massage, and diluted intralesional triamcinolone (5 mg/mL). In two of the three patients, the nodules persist 3.5 years later and are now ~50 to 80% resolved. Both patients have refused excision and are content to be treated with another product in this area that tends to hide the nodules.

Reported actual granuloma rates range from 0.2 to 12%.[59] Vleggaar[62] reported histologically confirmed granulomas in two patients (1.1% of his patients). The granulomas were treated in several ways, including intralesional steroids, subcision, oral steroids, and intralesional 5-fluorouracil (5-FU).[62] According to Lam et al,[59] nodules of PLA consist of the PLA particles surrounded by "normal" inflammation and arise within several weeks after the injection. They believe that granulomas are more ill defined, arise from the area of implantation months to years later, and can be, as explained earlier, subject to reactivation to a more acute inflammatory state years later.[4,5] In the opinion of Fulton et al,[63] the granulomas found with PLA and silicone are "similar" histologically. Certainly a granuloma that walls off a foreign body need not take years to develop.[4,5] Here again, as with CaHA, the distinction between a nodule and a granuloma seems to blur. It would be advantageous to have histologic examination of more nodules. It may be that many of the nodules are actually quiescent granulomas.

POLYMETHYLMETHACRYLATE MICROSPHERES IN BOVINE COLLAGEN

Whereas CaHA and PLA are marketed for semipermanent augmentation, polymethylmethacrylate (PMMA) microspheres suspended in bovine collagen is the first "permanent" facial filler approved by the FDA. Judet reported the first hip prosthesis made of PMMA in 1947.[64,65] PMMA is biocompatible and has been used widely over at least 3 decades as a cement in otolaryngology, neurosurgery, and joint replacement. PMMA is marketed in the United States as ArteFill. The FDA approved it in 2007.

The original product introduced in Europe, ArtePlast, had the same 30 to 42 μm diameter, but was in a gelatin carrier. The initial 587 subjects had a granuloma formation rate of 2.5% from 6 to 18 months after treatment. This high complication rate was believed to be secondary to the gel and to PMMA nanoparticle contamination inherent in the original manufacturing process. There were considerable numbers of particles small enough to be phagocytosed[64] but not transported away, resulting in chronic granuloma formation. An improved version of the product with more uniform distribution of size, much less nanoparticle contamination, and a collagen carrier was introduced in Europe in 1994. With this product, Artecoll, there has been noted a granuloma rate of less than 0.01% in more than 400,000 injections.[64]

The U.S. product, ArteFill, has less than 1% contamination with particles less than 20 μm. The PMMA particles are suspended in a water-based carrier gel that is 3.5% bovine collagen, 92.6% buffered isotonic water, 0.3% lidocaine, 2.7% phosphate buffer, and 0.9% sodium chloride.[66] There are ~6 million PMMA microspheres in 1 mL of ArteFill.[64] The bovine collagen functions as a carrier for implantation. As the collagen dissipates, the real augmentation with the microspheres persists. It is intended for injection into the deep dermis, not the subcutis.[2] With bovine collagen in the mix, the product can be very immunogenic. Double skin testing is required. The product requires refrigeration.

Clearly, it is reasonable to expect that the plastic microspheres are there for life and, hence, that the potential for granuloma formation may continue for life. As discussed earlier, size and smoothness are factors in phagocytosis. Particles less than 15 to 20 μm are generally able to be phagocytosed. Particles 30 to 42 μm are certainly large enough to resist initial phagocytosis by polymorphonuclear cells and require attack by macrophages. Morhenn et al.[11] demonstrated that PMMA particles 20 μm or less are likely to be phagocytosed. Microspheres of 40 to 50 μm are less likely to initiate an effective inflammatory reaction.[11] Also, all other dimensions being equal, a relatively smooth implant particle will be more resistant to phagocytosis than will a rough-surfaced particle, which presents many angles to the phagocytes. ArteFill microspheres are almost perfectly round. Because these spheres are very smooth, much smoother than silicone, it is reasonable to expect that these large, very smooth particles will be poorly phagocytosed. Hence, it is reasonable to expect PMMA to be resistant to phagocytosis and to tend to become encapsulated with a thick fibrous capsule. As with microdroplet deposits of silicone, it is the fibrous capsule that forms around the PMMA particle that actually accounts for the augmentation.[67]

Here, too, nodules can be an issue. Bagal et al[68] reported on 72 patients treated at 177 treatment sessions. Their opinion is that the lips are the area most susceptible to complications. With 34-month follow-up, they reported lip nodules in four patients and prolonged lip pain in one patient. They reported no biopsy results.

The reports of late, active granulomas with PMMA are not infrequent. Lemperle et al[67] reported an overall granuloma rate of 1:10,000 (0.01%), most occurring 1 to 2 years later. In this scenario, granuloma formation with encapsulation and the possibility of late reactivation is still a real possibility. In contrast with the assumption that PMMA is well tolerated and easily walls off, however, McClelland et al[69] found that that the PMMA microspheres in collagen (in this case Artecoll) were susceptible to phagocytosis in guinea pigs. Cohen et al[64] reported on the eight-center U.S. clinical trials of ArteFill in 2006. Of the 251 patients treated, 128 received ArteFill and 123 received collagen as a control. Sixty-nine patients returned for 4- to 5-year follow-up. Photographic examination of the patients examined at 4 to 5 years actually showed further improvement over what was seen at 3 months to 1 year after end of therapy. This might support the proposition that collagen neogenesis continues in the long term.[64] They also reported six late adverse events in five patients, four mild and two severe (3.9% of patients), 2 to 5 years after therapy. Two of these reactions were in one patient in the 4- to 5-year follow-up group. Both reactions were rated as severe. The patient had some nodularity in the melolabial folds and was treated with steroid injections. The four patients with mild adverse reactions had only lumps, with none in the lips. One required excision, but no pathology is reported. Reisberger et al[70] reported active foreign-body granulomas in the forehead of a 61-year-old woman injected with Artecoll 6 years prior. They treated the patient with allopurinol. And finally, in Brazil there are at least three different formulations of PMMA available on the market.[71] Artecoll (32 to 40 μm) has been replaced by ArteFill (32 to 40 μm). Metacrill (Nutricell Laboratory, Rio De Janiero, Brazil) (40 to 60 μm) and New Plastic (30 to 103 μm), which is commonly used in the technique of Bioplasty, use different carriers and are manufactured in Brazil. Also, until 2008 it was also possible to obtain PMMA compounded by "local handling pharmacies." Salles et al,[71] from Brazil, reported on 32 complications of PMMA injections in patients referred to their center over a 5-year period. The complications included tissue necrosis (5), granulomas (10), chronic nonspecific inflammatory reactions (10), lip stiffness, lymphedema, or nodules (6), and infection (1). The cases of tissue necrosis occurred acutely and included the penis, lip, melolabial fold, and the nose. The patients with granulomas and chronic inflammation received Artecoll, Metacrill, or had Bioplasty. The patients diagnosed with granulomas had nodules and localized pain at the site of the injection. Three of the granuloma cases were confirmed by biopsy, showing the PMMA particles surrounded by epithelioid cells, sclerotic collagen bundles, and multinucleated giant cells. The chronic inflammatory cases began 1 to 10 years after injection and were cyclic in nature. No biopsies were done on these patients and no nodules noted. The lip complications occurred 1 month to 6 years after injection, and the one infection occurred 1 year after injection. Certainly, the larger PMMA particles may have played a role in the complications. Also, differences in manufacturing and compounding controls may have led to products with perhaps significant contamination with other substances or nanoparticles. Even though this article presents a small sample for each PMMA filler and does not specify which filler was used in all cases, it is still certainly an humbling article. Complications with PMMA are rare but can be significant and devastating.

In addition, biofilm formation around orthopedic implants bonded with PMMA cement is a well-recognized risk.[72] Given the relatively early results for ArteFill as a facial filler and given the history of recurrent inflammatory reactions with silicone and risk of infection with PMMA cements, careful ongoing follow-up of patients treated with PMMA is certainly in order.

MANY UNANSWERED QUESTIONS

Is the potential for granulomatous inflammation the single most significant biologic characteristic of all facial fillers? Some products rely on fibrosis and collagen neogenesis for their effect. What is it that pushes this controlled granulomatous inflammation over the edge? With or without a capsule, does the mere presence of foreign-body giant cells and an increased number of fibroblasts around an implant define a clinically significant granuloma; or need there be clinical evidence of recent active inflammation? Does the potential for uncontrolled early and late granulomatous inflammation hang like the sword of Damocles over every treatment? Are we really just splitting hairs to distinguish between a quiescent granuloma and a "nodule"? It would certainly be helpful if there were more biopsies reported on what are assumed to be just "nodules."

Is the choice of which filler to recommend driven more by market forces than by science? Does a reluctance to be critical of a product that is safe and predictable in the vast majority of cases and specifically requested by patients lead us away from the objective science? Alternatively, does a physician's normal level of skepticism lead to the outlook that every nodule with a macrophage infiltrate, increased lymphocytes, and giant cells is a potentially dangerous granuloma? Any product can form a granuloma. Extremely low rates of granuloma formation still do not equal zero. No matter how low the

reported granuloma rate may be for a particular long-term filler, that rate is irrelevant to the patient who gets a painful or disfiguring granuloma.

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