

Section 3

COLLAGEN FILLERS

CHAPTER 13

Review of Collagen Fillers

Andrew B. Denton

Nael Shoman

Soft tissue augmentation dates back more than 100 years, and over the past few decades, many agents and techniques have been introduced to cosmetically enhance soft tissue defects. With more patients now seeking aesthetic improvements without major surgery, the emphasis on soft tissue augmentation has received widespread acceptance among patients and physicians. With aging, reduced subcutaneous fat and dermal collagen results in soft tissue volume depletion, which may be superficial, as with facial rhytids, or involve deeper planes.

Collagen is the major insoluble fibrous protein in connective tissue and is the most abundant protein in the body. It provides the major structural component of the dermis, comprising 70% of dry skin mass. There are at least sixteen types of collagen, each denoted by a Roman numeral. Eighty to 90% of the collagen in the body consists of types I, II, and III. Type I collagen was the first to be isolated and characterized. Its fundamental structural unit is a long (300 nm), thin (1.5 nm diameter) protein that consists of three coiled subunits: two $\alpha 1$ chains and one $\alpha 2$ chain. Each chain contains 1,050 amino acids wound around one another in a characteristic right-handed triple helix. The collagen triple-helical structure contains an abundance of three amino acids, glycine, proline, and hydroxyproline, making up the characteristic repeating motif Gly-Pro-X, where X can be any amino acid. Collagen is synthesized by fibroblasts as α procollagen, and the posttranslational hydroxylation of proline residues is performed by propyl hydroxylase in the presence of ascorbic acid (vitamin C). About 96% of the chain length is helical, with nonhelical telopeptides at the amino- and carboxylter-

mini. These telopeptides contain important antigenic loci. Hydrogen bonds linking the peptide bond NH of a glycine residue with a peptide carbonyl (C=O) group in an adjacent polypeptide help hold the three chains together. Many three-stranded type I collagen molecules pack together side by side, forming fibrils with a diameter of 50–200 nm. These fibrils, roughly 50 nm in diameter and several micrometers long, are packed side by side in parallel bundles, called *collagen fibers*. All collagens were eventually shown to contain three-stranded helical segments of similar structure; the combination of 3 α chains determines the type of the resulting collagen molecule.

During the embryonic period, collagen type III predominates in human skin. After birth, the ratio of collagen type I increases. Dermal collagen in adult skin is composed of type I (80 to 85%) and type III (10 to 15%), in addition to glycosaminoglycans and elastin fibers. Type I collagen remains the predominant type during childhood and early adulthood, but the proportion of type III will increase with aging. Overall, collagen production by fibroblasts decreases with age, accounting for an overall 20% reduction in dermal thickness in aged skin. Ultraviolet radiation will increase the level of matrix metalloproteinases (e.g., collagenase), which will accelerate collagen degradation and skin aging.

The first attempt at soft tissue augmentation was in 1893, when Neuber transplanted fat from the arm into facial defects. In 1899, Gersuny became the first to use an injectable material for a cosmetic deformity, when he injected paraffin into a patient's scrotum to create a testicular prosthesis. Although paraffin initially gained popularity as a filler material, the associated risk of foreign body

granulomas resulted in abandonment of its use in the 1920s. Liquid silicone was later developed in the 1960s as a filler agent and was utilized widely for superficial and deep soft tissue deformities, despite lack of Food and Drug Administration (FDA) approval. Its use, however, was burdened with risks of permanent beading, migration, and granuloma formation. Silicone was largely abandoned in the early 1980s. Because of the obvious defect in collagen in aged skin, the use of collagen as a filler became popular with the introduction and FDA approval of Zyderm, a bovine collagen, in 1981. An abundance of agents were introduced over the past two decades in a continuing quest for the ideal dermal filler (Table 13.1).

XENOGENIC MATERIALS

Bovine collagen was first extracted from fresh calf skin in 1958 by Gross and Kirk at the Harvard Medical School. They later demonstrated that under physiologic conditions, collagen has the property of precipitating into a rigid gel composed of fibrils with the characteristic axial periodicity of native collagen. Studies in the 1960s demonstrated that the major immunogenic sites in native tropocollagen are the telopeptides, and as such, selective removal of the nonhelical amino- and carboxylterminal segments of the collagen molecule significantly reduced its antigenicity. Investigators at Stanford University began work in the early 1970s on developing a clinically useful collagen implant material. In 1977, they injected purified human, rabbit, and rat collagen into rats and studied the evolution of the implants over time using light and scanning electron microscopy. In that same year, they published the results of their initial clinical trials using allogenic and xenogenic (bovine) collagen injection in twenty-eight patients, involving more than six hundred individual injections. Of twenty-eight patients, there was moderate to profound improvement in twenty-four, with follow-up periods exceeding one year.

Injectable bovine collagen was first introduced to the market as Zyderm (Collagen Corp.) in 1976 and was approved by the FDA in 1981 for the treatment of facial lines and wrinkles. It is a purified, enzyme-digested bovine collagen with the telopeptide regions of the molecule removed to reduce product antigenicity. The collagen is extracted from the hides of a closed American herd to protect against the possibility of bovine spongiform encephalopathy virus or prion contamination. Zyderm I, the initial product released, comprised 96% type I collagen, with the remainder being type III collagen, suspended in phosphate-buffered physiologic saline with 0.3% lidocaine. It is 3.5% bovine dermal collagen by weight. Due to quick resorption of the collagen, Zyderm II was thereafter introduced with an increased collagen concentration of 6.5% bovine dermal collagen by weight. Zyderm II gained FDA approval in 1983.

In an effort to produce a material with a longer-lasting effect, the collagen in Zyderm was cross-linked through glutaraldehyde processing by forming covalent bridges between 10% of available lysine residues, effectively inhibiting degradation by collagenase. The resulting product, Zyplast, was FDA approved in 1985. It is 3.5% bovine dermal collagen by weight and is more viscous, more resilient to biodegradation, and less immunogenic than Zyderm.

Zyderm and Zyplast are prepackaged in 1- or 2-mL syringes, and Zyderm II is packaged in 0.5-mL syringes; all are administered through a small-gauge needle. The products are stored at low temperature (4 degrees Celsius) and consolidate into a solid gel once implanted at body temperature. Zyderm I is injected into the superficial papillary layer, Zyderm II into the middermis layer, and Zyplast into the deep dermis. Zyderm I and II are used for correction of soft distensible lesions in the face with relatively smooth margins. These would include glabellar, forehead, and periorbital rhytids as well as fine perioral rhytids, acne scars, traumatic and surgical scars, and steroid-induced atrophy. Zyderm II has more collagen and is therefore more effective in moderate to deep wrinkles. Zyplast is more valuable in treating deeper lines often unresponsive to Zyderm such as nasolabial folds, deep acne scars, and the vermilion border of the lips. With Zyderm I, overcorrection by 100% is required as it is diluted with phosphate-buffered physiologic saline, which is reabsorbed typically over two to three months. With Zyderm II, 50% overcorrection is recommended. No overcorrection is required with Zyplast. As the saline is absorbed, host connective tissue cells grow into the collagen, giving the appearance of normal tissue. Eventually, the injected collagen is detected as a foreign substance, with an ensuing inflammatory response. Overall, results, albeit variable and dependent on the facial anatomic region, injection depth, and agent used, typically last two to three months for Zyderm, with Zyplast lasting about three to four months.

Prior to the administration of these products, intradermal skin testing is required. Approximately 3 to 5% of people will display a delayed hypersensitivity response to the skin challenge, generally by forty-eight to seventy-two hours. 1 to 2% of people may be sensitized following the skin challenge, and as such, many practitioners as well as the American Academy of Dermatology recommend a second skin test a few weeks following the first. Repeated injections may result in the development of hypersensitivity to bovine collagen. In general, skin testing is necessary for any patients new to bovine collagen as well as for patients who have not received treatment with bovine collagen for over one year.

Bovine collagen injections are contraindicated in patients undergoing treatment with steroids, in those with a history of allergy to other bovine products or meat or to lidocaine, or in those with a history of an autoimmune

TABLE 13.1: Summary of Collagen Fillers

Name	FDA	Ingredient	Duration of Effect (months)	Allergy Tests Needed	Storage: Shelf Life	Sizes Available	Rhytid Size: Indicated	Degree of Overcorrection
Zyderm I	yes	bovine collagen	3-4	two skin tests 2-4 weeks apart; may treat 4 weeks after second skin test	35 mg/mL; refrigerated 3 years	0.5, 1.0, and 1.5 cc syringes	fine lines	1.5-2.0 times
Zyderm II	yes	bovine collagen	3-4	two skin tests 2-4 weeks apart; may treat 4 weeks after second skin test	65 mg/mL; refrigerated 3 years	0.5 and 1.0 cc syringes	fine lines	1.5-2.0 times
Zyplast	yes	cross-linked bovine collagen	3-5	two skin tests 2-4 weeks apart; may treat 4 weeks after second skin test	35 mg/mL; refrigerated 3 years	0.5, 1.0, 2.0, and 2.5 cc syringes	large folds	no overcorrection
Cosmoderm I	yes	human collagen	3-4	no skin test	35 mg/mL; room temperature shelf life unknown	1.0 cc syringes	fine lines	100% overcorrection
Cosmoderm II	yes	human collagen		no skin test	65 mg/mL	0.5 cc only	fine lines	
Cosmoplast	yes	cross-linked human collagen	3-4	no skin test	35 mg/mL; refrigerated 3 years	1.0 and 1.5 cc syringes	large folds	no overcorrection
Autologen	yes	autologous collagen fibrils, elastin, fibronectin, and glycosaminoglycans	4-9	no skin test	50-120 mg/mL; frozen (kept with manufacturer) 5 years	3 mL syringes	moderate to deep defects	20% to 30% overcorrection
Isolagen	no	autologous cultured fibroblasts	unclear	test dose done 2 weeks prior to treatment	frozen (kept with manufacturer)	1-1.5 mL syringes	superficial to moderate defects	
Dermalogen	no	cadaveric suspension of types I and III collagen				1.0 mL syringes		20% to 30% overcorrection
Alloderm	yes	allogenic matrix of collagen, elascin, and glycosaminoglycans	12-24		sheets of different sizes (1 x 2 cm; 4 x 12 cm); room temperature 2 years	sheets	deep defects and scars	up to 200% overcorrection
Cymetra	yes	injectable form of Alloderm	3-6		330-mg powder in 5-cc syringe; room temperature 2 years	5 cc syringes	deep defects and scars	30% overcorrection
Fascian	yes	allogenic preserved particulate fascia	3-6	contraindicated with gentamicin allergy	80 mg/2 mL; room temperature 2 years	0.25, 0.5, 1.0, and 2.0 ug particles; 80 mg in each 3 cc syringe	deep defects and scars	
Evolence	no	cross-linked porcine collagen	12	no skin test	35 mg/ml; room temperature 3 years	1.0 cc syringe	moderate to deep lines and folds, lips	no overcorrection

disorder, particularly a collagen vascular disease. Zyplast is contraindicated for the treatment of glabellar rhytids due to the possibility of central retinal artery occlusion. Bruising, scarring, and infection may appear postinjection. Localized necrosis occurs with an incidence of 0.09%, with the glabellar area at higher risk due to its dependence on the supratrochlear vessels for perfusion. Reactivation of herpes is possible with lip injections, and as such, patients with a positive history need antiviral prophylaxis. Approximately 0.5% of patients may experience systemic symptoms with flulike symptoms, paresthesias, or difficulty breathing.

BIOENGINEERED HUMAN COLLAGENS

The human-derived collagens Cosmoderm and Cosmoplast were FDA approved in March 2003 for cosmetic indications and contain types I and III human bioengineered collagen. They do not require skin tests prior to treatment.

Dermal fibroblasts are harvested from bioengineered human skin cells qualified by extensive testing for viruses, retroviruses, cell morphology, karyology, isoenzymes, and tumorigenicity. The cells are seeded onto a three-dimensional mesh that is then cultured in a bioreactor under conditions simulating those found in the human body. The fibroblast cells attach to the mesh within the reactor, replicate, and secrete collagen and extracellular proteins. The developing dermal tissue on the mesh is identical to the human dermis but lacks immunological cells and melanocytes. The extracellular type I and type III collagen is isolated from the resulting dermal tissue and is capable of binding with hyaluronic acid and other molecules to provide structure to the skin. As with Zyplast, Cosmoplast has gluteraldehyde cross-linking the lysine residues to make it more resilient to degradation by collagenase.

Cosmoderm I contains 35 mg/mL of collagen dispersed in a phosphate buffered saline solution and 0.3% lidocaine. Cosmoderm II is similar but contains about twice the collagen concentration. Cosmoplast is similar to Cosmoderm I but is cross-linked with gluteraldehyde. These products are prepackaged in 1-mL syringes and should be refrigerated (4 degrees Celsius) but not frozen. They are typically injected using a 30-gauge needle. The manufacturer recommends administration with an accurate depth gauge assist device on the needle, adjusted so that a 2-mm length of the needle tip is exposed.

Cosmoderm is injected into the superficial papillary dermis, and Cosmoplast is injected into the mid- to deep dermis. Cosmoderm is best suited for correcting fine lines and acne scars, and Cosmoplast is best suited for the correction of deeper rhytids, for smoothing scars, and for lip enhancement. Overcorrection by 150 to 200% is necessary with Cosmoderm because it is diluted with saline, which is reabsorbed. A lesser degree of overcorrection is needed

for Cosmoderm II than for Cosmoderm I, and no overcorrection is needed with Cosmoplast. Results seen with these products are immediate, and the duration of effect is typically three to six months.

The use of these products is contraindicated in patients with a lidocaine allergy, and they should be used cautiously in patients with autoimmune diseases such as rheumatoid arthritis.

AUTOLOGOUS HUMAN COLLAGENS

In the late 1980s, a research and development biomedical lab (Autogenesis Technologies) investigated the feasibility of extracting intact human collagen fibers for injection from skin obtained electively during aesthetic plastic surgical procedures. Results reported with injectable autologous collagen varied, and further development of this product resulted in Autologen (Collagenesis Inc.).

Autologen is a dermal matrix dispersion derived from the patient's own skin, predominantly composed of intact collagen fibrils of types I, III, and IV. Processing of the specimen allows for isolation of collagen fibrils, elastin, fibronectin, and glycosaminoglycans. Syringes are returned to the physician after three to four weeks. Unlike bovine collagen, which is treated to remove the telopeptide allogenic units, Autologen contains only human proteins, and no such treatment is required. The collagen concentration ranges from 50 to 120 mg/mL. Only a limited amount of collagen tissue persists with each injection, and usually, three or more injections are given over a period of several weeks to fully correct most dermal defects. Overcorrection of approximately 30% is recommended during each treatment. The tight collagen fibrils and lack of xenoproteins are thought to result in minimal inflammatory response, with tissue augmentation relying mostly on the injected collagen. On average, 1.0 mL of 5% Autologen requires 2 square inches of skin (excluding skin obtained from blepharoplasty). Harvested skin can be stored at Collagenesis BioBank if immediate use is not needed or uncertain. Autologen is available in 1.0-mL syringes and is stored under refrigeration for up to six months.

Skin testing is not needed with the use of Autologen. Its autologous nature further eliminates the potential for donor-to-recipient disease transmission. While the concept of Autologen may be very attractive for patients undergoing skin excision procedures, those who are not must subject themselves to a skin harvesting procedure. The injectable material is not prepared with lidocaine and so the injections are more painful, and may necessitate nerve blocks or topical anesthesia. Autologen augmentation can last three to six months; however, a recent study showed no statistically significant difference between Autologen and Zyplast in implant persistence over a twelve-week period.

ALLOGENIC HUMAN COLLAGENS

The advantages of Autologen compared to other intradermal injectable biomaterials were offset by the need for an elective surgical procedure during which skin was removed. As such, the search continued for an allogenic injectable human collagen available prepackaged from tissue donors. Central to this search is the concept that intraspecies collagen is identical, and as such, an economically available and safe allogenic collagen product could be harvested that is essentially identical to autologous collagen.

Dermalogen (Collagenesis Inc.) is a dermal filler derived from human cadavers at tissue banks accredited by the American Association of Tissue Banks. The skin is deepithelialized and aseptically processed to provide an acellular suspension of collagen fibers and human collagen tissue matrix. The product is structurally identical to Autologen, but without the need for the preliminary harvesting and prolonged processing. The donors' past medical and social histories are thoroughly reviewed; blood samples are taken for viral testing; and skin specimens are tested for bacterial, fungal, or viral contamination. A sterilization protocol is followed despite negative screening for known pathogens. The final product is supplied in boxes of six 1.0-mL syringes that can be refrigerated for six months.

Skin testing prior to treatment with Dermalogen is not required but is recommended to ensure that no hypersensitivity to the delivery vehicle or by-products of processing exists. Intradermal injections of Dermalogen supplied in separate containers for this purpose are typically administered to the volar forearm, and absence of a reaction at seventy-two hours excludes a hypersensitivity response. A single test is sufficient. Dermalogen is injected into the middermis, with 20 to 30% overcorrection recommended. The results with Dermalogen have been generally satisfactory, with a duration lasting approximately three to six months.

Alloderm (LifeCell Corp.) is an acellular processed human dermal allograft matrix derived from cadaveric skin at tissue banks and has been in use since 1992. A freeze-drying process eliminates all cells and leaves a matrix of collagen and dermal elements, including laminin, elastin, and proteoglycans. No class I or II histocompatibility antigens were shown using immunohistochemical staining. Alloderm has the capacity to incorporate into the surrounding tissue, acting as a framework to allow ingrowth of native tissue and allowing for revascularization. It is manufactured as sheets and, following rehydration, is inserted through an incision or dissected dermal tunnels. It may be used for treating full-thickness burns, surgical defects, and acne scars as well as for soft tissue augmentation. Alloderm sheet volume persistence is significantly greater than Zyplast during the first three months after placement. Overcorrection

is needed and will depend on the size of the defect, but up to 200% may be necessary.

Cymetra (LifeCell Corp.), available since 2000, is a micronized form of Alloderm for injectable use. Strips of homogenized Alloderm sheets are cut out, dried, and packed as 330 mg of product in 5-cc syringes that may be refrigerated for up to six months. The freeze-dried particles are mixed with 1 mL of lidocaine or saline up to two hours prior to injection. Once injected, host fibroblast and collagen ingrowth as well as neovascularization ensue. Overcorrection by 30% is recommended, and multiple treatments may be warranted. The duration of response is generally noted to be longer than bovine collagen, lasting approximately four to six months. As with Alloderm, skin testing is not required.

Fascia has been extensively utilized in the past for numerous applications, including suture material, dura repair, tendon and ligament repair, and tympanic membrane replacement. Clinical response persists for years following implantation, with minimal complications. Native collagen ultimately replaces implanted fascial grafts, as consistently demonstrated by histological studies. Dermatologic applications were first adopted by Burres in 1994, who utilized the material for volume replacement. His work on acne scars and, later, on lip augmentation led the development of preserved particulate fascia (Fascian, Fascia Biosystems), first distributed as an injectable filler material in 1998. The fascia in Fascian comes primarily from human cadaveric donor gastrocnemius fascia. Following processing through sterilized baths, the material is freeze-dried to a water content of less than 6%, and is then sterilized with gamma radiation and ethylene oxide. The graft is then particulated and loaded into vacuum-packed syringes. Syringes of five different particle sizes (0.1, 0.25, 0.5, 1, and 2 mm) are available, each containing 80 mg. Because the material is freeze-dried, it may be stored at room temperature for up to five years. Fascian particulate and 1.5–2.0 mL of 0.5% lidocaine are mixed in the syringes for one minute. The finer particles may be injected with a 20- to 25-gauge needle, but the larger particles may require a 14- to 18-gauge needle. No skin testing or refrigeration is required; however, trace amounts of polymyxin B, bacitracin, and/or gentamicin may be present in Fascian and may trigger a hypersensitivity response in allergic recipients. Injections should only be made in healed defects without active inflammation. Fascian may be injected intradermally, subdermally, or in the subcutaneous tissue. After injection, the graft matrix is digested over the ensuing several months. New collagen is then laid down by invading fibroblasts, a process termed *recollagenation* by Burres, and as such, preserved fascia grafts may result in longer-lasting tissue augmentation. Although the exact duration remains largely unknown, one study found Fascian still persistent three to four months following injection.

EVOLENCE AND EVOLENCE BREEZE

Evolve and Evolve Breeze (ColBar LifeScience Ltd.) are the latest products available in the evolution of collagen-derived fillers. Both Evolve and Evolve Breeze are produced from the same porcine collagen and are formulated at a collagen concentration of 35 mg/mL. The products differ in their rheologic properties, which are reflected in their viscosity and injectability properties. The viscosity of Evolve Breeze is approximately 60% of the viscosity of Evolve and is injectable through a 30-gauge needle. Evolve has been available in Europe and Canada for a couple years, and it appears to be a far superior product in terms of duration of results than the Zyplast/Cosmoplast products, and at least comparable to Restylane/Perlane, Juvéderm, and Radiesse. Evolve became available in the United States in late 2008, and Evolve Breeze is expected in early 2009. Both are discussed in more detail in Chapter 15. Table 13.1 summarizes collagen-based products.

SUGGESTED READING

- Baumann L, Kaufman J, Saghari S. Collagen fillers. *Dermatol. Ther.* 2006;19:134-40.
- Eppley BL, Dadvand B. Injectable soft-tissue fillers: clinical overview. *Plast. Reconstr. Surg.* 2006;118:98e-106e.
- Fagien S. Facial soft tissue augmentation with autologous and homologous injectable collagen. In: Klein AW, ed., *Tissue Augmentation in Clinical Practice: Procedures and Techniques*. New York: Marcel Dekker; 1998:88-124.
- Klein AW. Skin filling: collagen and other injectables of the skin. *Dermatol. Clin.* 2001;19:491-508.
- Knapp TR, Luck E, Daniels JR. Injectable collagen for soft tissue augmentation. *Plast. Reconstr. Surg.* 1977;60:398-405.
- Lowe NJ, Maxwell CA, Patnaik R. Adverse reactions to dermal fillers: review. *Dermatol. Surg.* 2005;31(11 Pt 2):1616-25.
- Matton G, Anseeuw A, De Keyser F. The history of injectable biomaterials and the biology of collagen. *Aesthetic Plast. Surg.* 1985;9:133-40.
- Skouge J, Diwan R. Soft tissue augmentation with injectable collagen. In: Papel I, Nachlas N, eds., *Facial Plastic and Reconstructive Surgery*. St Louis: Mosby Year Book; 1992:203-12.