

Understanding the **Biochemistry** of **Dermal Fillers**

Learning how the biochemistry of the product can enhance patient outcomes is an important step in setting and managing proper patient expectations.

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In our society, a youthful appearance is usually associated with smooth, wrinkle-free skin and equated with health and vigor. From 2006 to 2007, the American Society of Plastic Surgeons (ASPS) reported a four percent increase in all cosmetic procedures, with over 12 million surgical and non-surgical cosmetic procedures performed.⁸ Interestingly, while there was essentially no increase in surgical procedures, ASPS posted a seven percent surge in non-surgical, minimally-invasive procedures: 1.5 million procedures were surgical while the other 10 million were non-surgical. The total revenue generated from cosmetic procedures in 2007 was estimated to be over \$13 billion, four billion of which were from non-surgical procedures.⁸ This divide between surgical and non-surgical will likely only continue to widen, since in most cases minimally-invasive procedures are, by their very nature, safer than surgical procedures, less traumatic to the patient, require minimal hospitalization, if any, and, consequently, cost less while providing the patient with seemingly comparable aesthetic results. There are, of course, situations when surgery is the only option. Nevertheless, given current economic constraints and the availability of “lunch-time enhancement” procedures, the popularity of minimally-invasive aesthetic procedures is expected to rise.

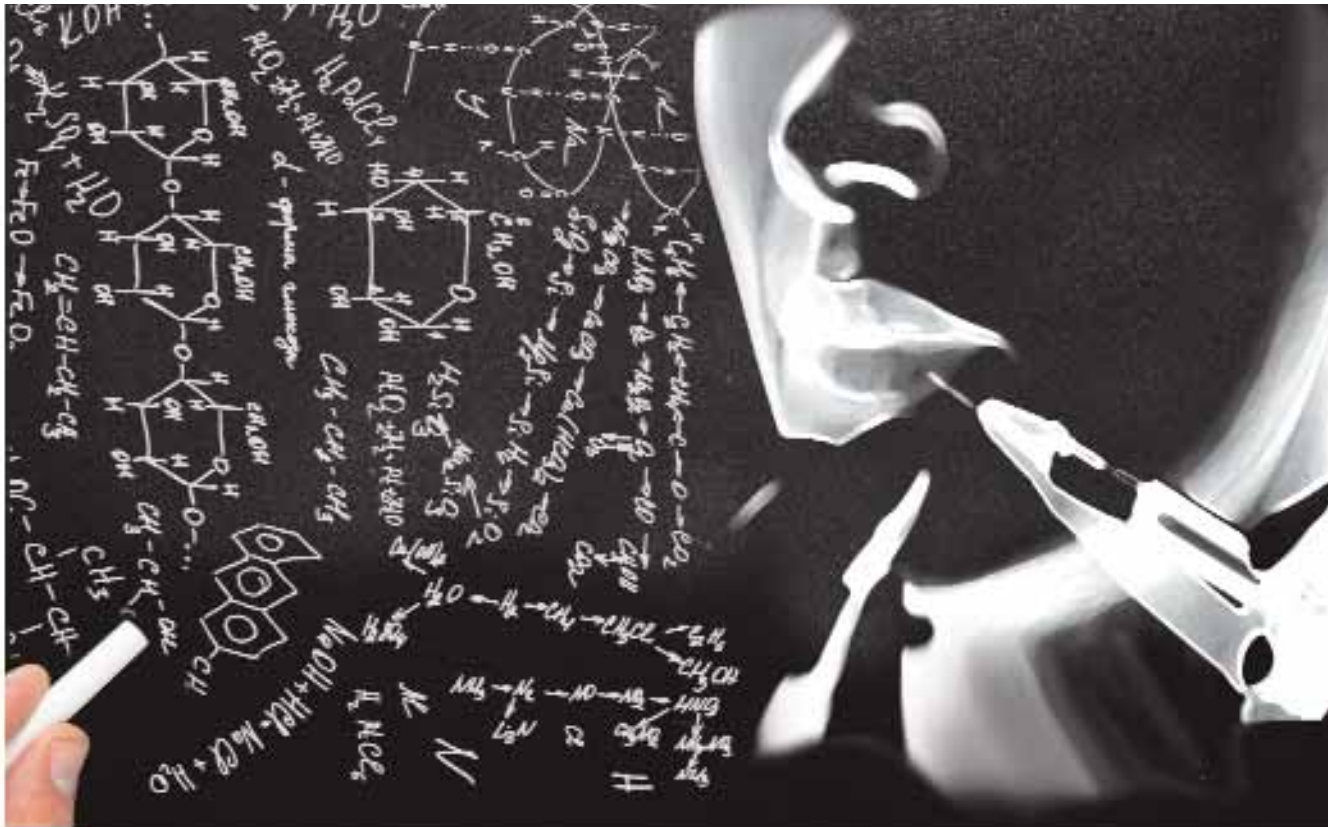
In order to protect cosmetic practice from the negative impacts of a down turned economy, greater emphasis should

be placed on the patient/provider relationship and savvy business model. When cash flow is limited, individuals are more likely to shop for the most economic pricing in lieu of consideration of customer service and provider loyalty. To enhance the quality of care they provide patients, physicians would benefit from a greater comprehension of the science of collagen fillers and hyaluronic acids. Understanding how the biochemistry of the product affects patient outcomes is an important step in setting and managing proper patient expectations. This article will provide a basic review of the biochemistry of type I collagen and hyaluronic acid dermal fillers.

Science 101

Collagens are a ubiquitous family of proteins comprised of at least 28 members.¹ Their unique, triple helix structure allows them to interact with cells and other extracellular matrix proteins resulting in signals that are essential for cell proliferation, differentiation, and survival. Type I collagen is the most abundant collagen in the human body comprising approximately 90 percent of the skeletons in mammals. In addition to bones, type I collagen is present in intervertebral disks, skin, granulation tissues, tendons and ligaments, cornea, dentine and arteries.^{2,3}

As people age, the structural integrity of the skin decreases due to degeneration of components, mainly collagen and



elastin, and a reduced capacity to regenerate.^{4,5} The collagen in the skin, along with other components, is degraded due to activation of matrix-metalloproteinases.^{6,7}

The two components most widely used in aesthetic dermal devices are collagen and hyaluronic acid (HA).^{9,10} Both are natural products and components of the skin, with type I collagen comprising 50 percent of the skin and HA one to two percent. Collagens were first to be used as fillers with the introduction of bovine collagen in 1981.¹¹ However, allergic reactions to the substance and other adverse events, such as local necrosis and abscess formation, soon decreased its popularity with patients and providers alike; skin testing was required prior to use.¹²⁻¹⁴ In addition, the technology of the first generation collagens was not able to provide extended duration.

In 2008, Dermicol P-35, a dermal filler composed of ribose cross-linked porcine collagen (Evolvece, Ortho Dermatologics) was introduced into the United States market, while it was widely available in Europe and Canada since 2005.¹⁷ Given the history of bovine collagen, the FDA required extensive testing to examine the possibility of type I and type IV allergic reactions. Two clinical trials were performed to examine the potential for type I and IV allergic reactions. In both pivotal trials no allergic adverse events were reported, negating the need for skin testing prior to use.¹⁴ In

addition to the safety profile findings, efficacy results were comparable to HA-based filler and lasted at least as long.^{18,19}

In the pivotal FDA non-inferiority, split face study, the data revealed fewer adverse events in the Dermicol P-35-treated side compared with the HA-treated side. It is important to note that the differences were not statistically significant, and the inclusion criteria, measurement tools, and grading scales were different.¹⁸

To evaluate the rate of patient-assessed adverse events (AE) reported immediately after a procedure using either hyaluronic acid (HA)-based and collagen-based dermal fillers, we searched PubMed using the terms “hyaluronic acid *or* collagen” and “filler.” We limited the search with the terms “English” and “humans.” A total of 173 publications were identified of which 23 were classified as clinical trials. Of these, only two provided details of patient-reported adverse events immediately after the procedure. The search was therefore augmented with the publicly available package inserts for these products, which also contained these data.

While the data for HA-based fillers is much more extensive than for Evolvece, and the single trial comparing Evolvece and Restylane did not show any statistical difference (see Table 1, next page), nonetheless there appears to be a trend toward a higher level of patient-reported AEs, such as bruising, when treated with Restylane than with Evolvece.

Table 1. Comparison of patient-reported adverse events immediately after procedure

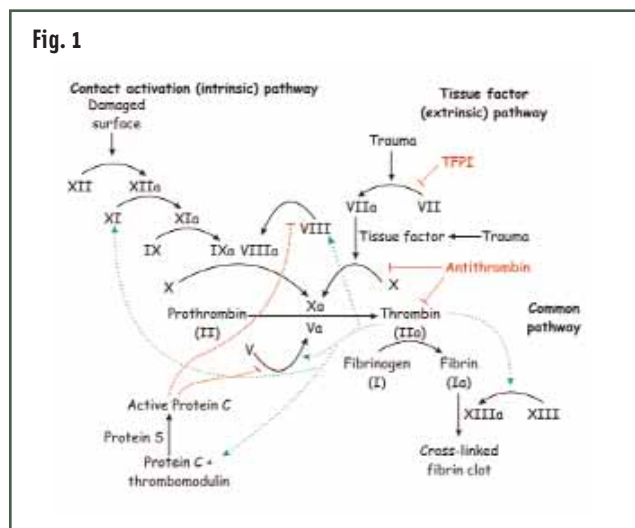
Filler	Number of Patients in Study	Patient-Reported Adverse Events								Ref. No.
		Redness		Swelling		Bruising		Pain		
		n	%	n	%	n	%	n	%	
<i>Data from clinical trials</i>										
Restylane®	138	117	84.8	120	87.0	72	52.2	79	57.2	21
Restylane®	149	91	61.1	101	67.8	72	48.3	95	63.8	18
Evolence®	149	88	59.1	78	52.3	56	37.6	85	57.0	18
<i>Data from package inserts</i>										
Restylane®	150	87	58.0	125	83.3	70	46.7	96	64	22
	142	114	80.3	127	89.4	111	78.2	108	76.1	
	137	117	84.8	120	73.9	72	52.2	79	57.2	
Perlane®	150	92	61.3	121	80.7	74	49.3	103	68.7	22
	142	118	78.2	128	90.8	122	86.5	114	80.9	
Juvederm™ Ultra	146	136	93.0	125	86.0	86	59.0	131	90.0	24
Juvederm™ Ultra Plus	144	129	90.0	124	86.0	87	60.0	129	90.0	25
<i>Mean Data</i>										
Restylane®			73.8		80.3		55.5		63.7	
Perlane®			69.8		85.8		67.9		74.8	
All HA			76.8		82.8		59.2		72.0	

Understanding Coagulation

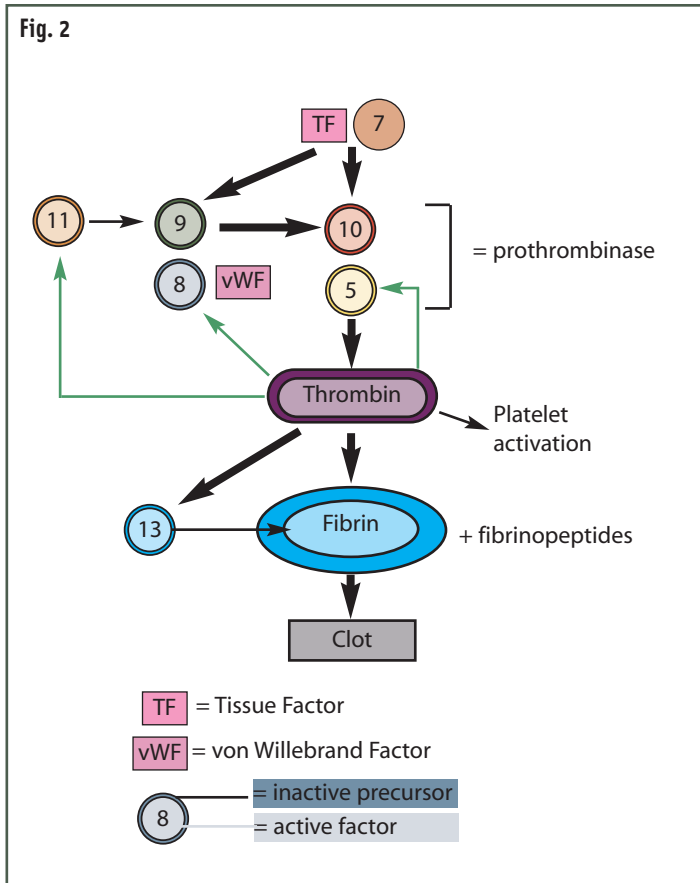
Coagulation is a dynamic and complex process and is essential to maintaining homeostasis. In all mammals, coagulation involves primary and secondary homeostasis, which occurs simultaneously. The human coagulation system is the most researched and currently is the most thoroughly understood. Primary (cellular) homeostasis involves the activation of platelets, and secondary homeostasis involves coagulation factors (proteins).¹

In a normal coagulation system, primary homeostasis starts when damage to a blood vessel wall exposes sub-endothelium proteins, most notably type I collagen, present under the endothelium. Circulating platelets bind to the collagen via surface collagen-specific glycoproteins Ia/IIa receptors. Additional strength is added to the adhesion through large, multimeric circulating proteins (clotting factors) in circulating blood plasma, most notably Von Willenbrand factor (vWF). The role of vWF is to provide the link between the platelets glycoprotein Ib/IX/V and the collagen fibrils. The formation of this adhesion results in activation of platelets.

Secondary homeostasis or the coagulation cascade is



described in two pathways (Fig. 1). The contact activation pathway, previously referred to as the intrinsic pathway, and the tissue factor pathway, previously referred to the extrinsic pathway, lead to the formation of fibrin. Previous research supported the theory that the two path-



ways were separate but equal pathways that merged together in a common pathway. Current understanding supports that the primary pathway for initiation of blood coagulation is the tissue factor pathway, which is the result of trauma, for example, trauma induced secondary to needle puncture.

Hyaluronic acid is a glycosaminoglycan (GAG) consisting of disaccharide units of D-glucuronic acid and N-acetylglucosamino-2-acetamido-2-deoxy-D-glucose and is connected by $\beta(1 \rightarrow 3)$ glycoside bonds. In its natural form, HA has a linear structure. In the manufactured form, HAs are cross-linked and have a molecular weight ranging from 50,000 to 8,000,000 Daltons or more depending on the source or formulation.

HA is found throughout the body, mainly within connective tissue, synovial fluid, vitreous humor of the eye, and in some bacteria. The uses of natural linear HA for dermal fillers and other medical applications are severely limited by in vivo degradation by enzymatic systems including: hyaluronidase, glucodisase, and glucuronidase. In order to combat the inherent problems associated with natural linear hyaluronic acid, cross-linking is essential. The technology of commercially available HA dermal fillers remain proprietary and not avail-

able to the public, but it is widely accepted that cross-linking and stabilization are pivotal to the success of the final product.

One of the technologies that is commonly used in the production of stabilized cross-linked hyaluronic acid is sulphation of heparines, hepranas and dermatans. Sulphation adds protection to the cross-linked HA by making it resistant to enzymatic degradation. Sulphation or supersulphation of glycosaminoglycans such as with heparin, dermtan sulphate, chondroitin is known to increase their anti-coagulation properties through the inhibition or changing of their ratio of factors Xa and IIa from the starting product. In addition, the ability to absorb water is dependent on the degree of cross-linking and sulphation. The sulphation of HA demonstrates anti-coagulant activity but more suprisingly a lack of platelet activation and aggregation as measured by the anti-adhesvie activity P.R.P model in rabbits when subjected to behavioral stress. Furthermore, this effect is totally absent in the natural liner hyalarouic acid.²⁶

Dermal fillers using HA became more popular since there was little, if any, immunological reaction to them.¹⁵ While HA is a hygroscopic material that absorbs water over time and can lead to overcompensation of the region, resulting in a swollen appearance, this effect can be corrected biochemically in a follow-up visit by injecting hyaluronidase, an enzyme that degrades HA.¹⁶ Although HA-based fillers are currently the most popular, there are opportunities for improvement. It is our observation that some patients treated with HAs have more bruising, which extends past the area of treatment.

Case Studies: Bruising

Case Study 1. A photograph (next page) was submitted by a patient that had hylauroinc acid injection in her lips and mental crease at 24 hours post-injections. Pinpoint bruising was noted during the procedure. Interestingly, the bruising had extended past the point of injection in the mental crease and nasal labial fold (Fig. 3).

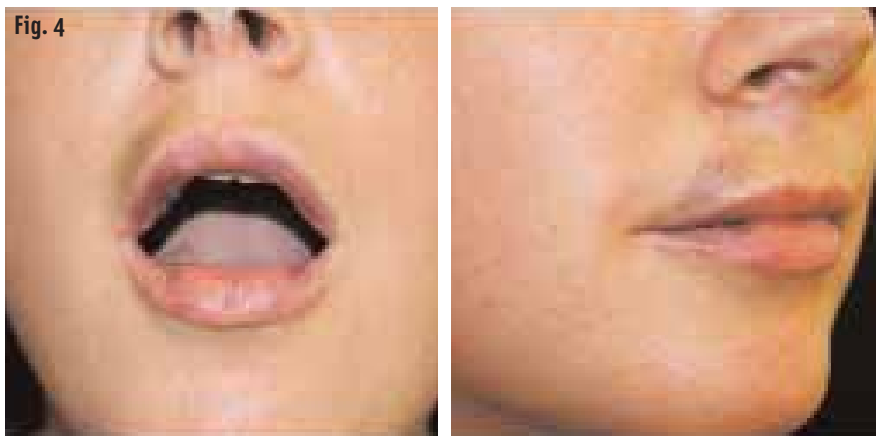
Case Study 2. This patient was injected with HA in the lip. No product or injection was placed above the vermilion border. She returned to clinic 24 hours later for follow up (Fig. 4).

Observing these cases, our hypothesis was that type I collagen, unlike HA, has a key role in coagulation that may account for less bruising and decreased blood loss from wounds through activation of platelets locally.²⁰ Furthermore, we postulate that HAs possess a degree of anticoagulation properties that may

Biochemistry of Dermal Fillers



Case 1. The patient above had been treated with HA injection in her lips and mental crease. She submitted the photo on the left at 24 hours post-injections. The photo on the right was taken 72 hours post-injection.



Case 2. The patient above was injected with HA in the lip. No product or injection was placed above the vermilion border. Photos taken at 24-hour clinical follow-up.

account for increases in bruising and involvement in tissues past the point of injection or correction.

In two separate dermatology practices with combined clinical experience in over 200 patients using Dermacol P-35, it was observed that patients had less bruising and swelling. This clinical experience is supported by the summary of post procedure adverse event data with most notable difference in bruising and swelling. To further investigate this phenomenon, we performed basic bench science testing to examine the effect of type I collagen on clotting time.

Methods. We obtained 30ml of blood through antecubital venipuncture from a healthy individual in three separate 10ml red top chemistry tubes free of additives at two

different clinical sites. At the same time, either 1ml of Dermacol P-35 or 1ml of commercially available hyaluronic acid was added to two separate vacutainers. The third vacutainers were used as the control. All three vacutainers were then continuously inverted for 60 minutes and the time was recorded to clot formation.

Results. In both sites, results were consistent. The red top tube containing Dermacol P-35 was the first to have clot formation, followed by the control tube. The HA tube did not show clot formation at 60 minutes. (See Table 2)

At site A, the vial containing Dermacol P-35 had partial clotting noted at 02:10. Complete clotting was observed at 03:45. In contrast, the red top tube containing the hyalauronic acid showed no signs of clotting at the end of study (60 minutes). The control tube containing whole blood had had clot formation at 37:20 with normal clot retraction beginning at 60 minutes.

At site B, the vial containing Dermacol P-35 had partial clotting noted a 02:32 seconds. Complete clotting was observed at 04:15. The tube containing the hyalauronic acid did not have clot formation at the end of study (one hour). The control tube containing whole blood had clot formation at 35:10 with no clot retraction noted at 60 minutes.

Conclusion. The data we obtained through this simple bench experiment supports our hypothesis and clinical experience. It lends further support to the adverse event data from the Dermacol P-35 pivotal trial. In order to fully appreciate the affects that collagen and hyalauronic acid have on coagulation, further testing must be conducted.

Chemical to Clinical Differences

Setting and managing patient outcomes and expectations are integral to developing customer satisfaction. Both FDA-approved fillers have different properties that can affect patient outcomes. Understanding the nuances of each dermal filler will help to minimize adverse events and maximize

Table 2. Results

Time to clot formation	Dermacol P-35 (Evolve®)	Hyaluronic Acid (Restylane®)	Control Whole Blood
Site A	03:45	None noted at 60 minutes	37:20
Site B	04:15	None noted at 60 minutes	35:10

patient outcomes. Dermacol P-35 is a sugar cross-linked collagen with a triple helix structure that may be associated with less bruising and swelling. But it is important to clearly set the patient's expectations that unlike the HA gels, collagen is much more structural in nature with the result that it is more easily palpable for longer period of time. Without coaching and education, the provider runs the risk of the patient confusing the palpability of the product with lumps and bumps.

On the other hand, HAs appear to have the potential to increase bruising due to their anticoagulation properties. They provide correction via hygroscopic effects, which equates to edema. Therefore, it is important to educate the patient that the acute inflammatory response will subside. The product will soften after the first several weeks. Treatment may be more "correctible" in the short-term thanks to the availability of hyaluronidase.

Another Layer

Importantly, while this discussion provides another vantage point for understanding and using dermal fillers, it serves as a stepping stone for a larger dialogue about minimally invasive procedures that is only in its beginning stages. Research on coagulation is relatively new with regards to dermal fillers, but our findings suggest that it represents an important area of discussion and inquiry. If anything, the information provided here will provide physicians with a better perspective on collagen-based and hyaluronic acid fillers that will aid clinical judgments. ■

Ms. Hanna is on the speaker bureau for Astellas and is a consultant and on the speaker bureau for Johnson & Johnson.

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Ms. Yubas has no disclosure.

- Heino J. The collagen family members as cell adhesion proteins. *Bioessays* 2007;29:1001-10.
- Pierschbacher MD, Polarek JW, Craig WS, et al. Manipulation of cellular interactions with biomaterials toward a therapeutic outcome: A perspective. *J Cell Biochem* 1994;56:150-4.

3. Leitinger B, Hohenester E. Mammalian collagen receptors. *Matrix Biol* 2007;26:146-55.

4. Makrantonaki E, Zouboulis CC. William J. Cunliffe scientific awards. Characteristics and pathomechanisms of endogenously aged skin. *Dermatology* 2007;214:352-60.

5. Calleja-Agius J, Muscat-Baron Y, Brincat MP. Skin ageing. *Menopause Int* 2007;13:60-4.

6. Rittié L, Fisher GJ. UV-light-induced signal cascades and skin aging. *Ageing Res Rev* 2002;1:705-20.

7. Baumann L. Skin ageing and its treatment. *J Pathol* 2007;211:241-51.

8. American Society of Plastic Surgeons. 2000/2006/2007 national plastic surgery statistics: Cosmetic and reconstructive procedure trends. 2008; www.plasticsurgery.org/media/statistics/loader.cfm?url=/commonspot/security/getfile.cfm&PageID=29287. Accessed on December 2, 2008.

9. Baumann L. Dermal fillers. *J Cosmet Dermatol* 2004;3:249-50.

10. Baumann L, Kaufman J, Saghari S. Collagen fillers. *Dermatol Ther* 2006;19:134-40.

11. Klein AW. Collagen substances. *Facial Plast Surg Clin North Am* 2001;9:205-18, viii.

12. DeLustro F, Smith ST, Sundsmo J, et al. Reaction to injectable collagen: Results in animal models and clinical use. *Plast Reconstr Surg* 1987;79:581-94.

13. Hanke CW, Higley HR, Jolivet DM, et al. Abscess formation and local necrosis after treatment with Zyderm or Zyplast collagen implant. *J Am Acad Dermatol* 1991;25:319-26.

14. Shoshani D, Markovitz E, Cohen Y, et al. Skin test hypersensitivity study of a cross-linked, porcine collagen implant for aesthetic surgery. *Dermatol Surg* 2007;33 Suppl 2:S152-8.

15. Clark CP, 3rd. Animal-based hyaluronic acid fillers: Scientific and technical considerations. *Plast Reconstr Surg* 2007;120:275-325.

16. Hirsch RJ, Brody HJ, Carruthers JD. Hyaluronidase in the office: A necessity for every dermasurgeon that injects hyaluronic acid. *J Cosmet Laser Ther* 2007;9:182-5.

17. ColBar LifeScience L. Evolve® collagen filler. 2008; www.evolve.com/us/safety-information-us.jsp;jsessionid=F5XWNAGEcX3DbHPU8G815F0KnNg. Accessed on October 6, 2008.

18. Narins RS, Brandt FS, Lorenc ZP, et al. A randomized, multicenter study of the safety and efficacy of Dermicol-P35 and non-animal-stabilized hyaluronic acid gel for the correction of nasolabial folds. *Dermatol Surg* 2007;33 Suppl 2:S213-21; discussion S21.

19. Narins RS, Dayan SH, Brandt FS, et al. Persistence and improvement of nasolabial fold correction with nonanimal-stabilized hyaluronic acid 100,000 gel particles/ml filler on two retreatment schedules: Results up to 18 months on two retreatment schedules. *Dermatol Surg* 2008;34 Suppl 1:S2-8; discussion S8.

20. Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol* 2008;28:403-12.

21. Narins RS, Brandt F, Leyden J, et al. A randomized, double-blind, multicenter comparison of the efficacy and tolerability of Restylane versus Zyplast for the correction of nasolabial folds. *Dermatol Surg* 2003;29:588-95.

22. Medicis Aesthetics Inc. Restylane®. 2008; <http://www.restylaneusa.com/instruction-use.html>. Accessed on October 27, 2008.

23. Medicis Aesthetics Inc. Perlane®. 2008; <http://www.restylaneusa.com/instruction-use.html>. Accessed on October 27, 2008.

24. Allergan Inc. Juvederm™ ultra. 2008; <http://www.juvederm.com/professionals/clinical.aspx>. Accessed on October 27, 2008.

25. Allergan Inc. Juvederm™ ultra plus. 2008; <http://www.juvederm.com/professionals/clinical.aspx>. Accessed on October 27, 2008.

26. Abstract IL 15, International Conference on Advances in Biomaterials and Tissue Engineering, Capri Italy, June 1998.