

# Rheological characteristics of hyaluronic acid-based dermal fillers before and after flowing through needles

*Características reológicas de preenchedores dérmicos à base de ácido hialurônico antes e após passagem através de agulhas*

## Authors:

Adilson Costa<sup>1</sup>  
Livia Carolina Della Coletta<sup>2</sup>  
Aline Siqueira Talarico<sup>2</sup>  
Maria Carolina Fidelis<sup>2</sup>  
Ellem Tatiani de Souza Weimann<sup>2</sup>

<sup>1</sup> PhD in Dermatology from the Faculdade de Medicina da Universidade de São Paulo (FMUSP)—São Paulo (SP), Brazil; MSc in Dermatology from Universidade Federal de São Paulo (UNIFESP)—São Paulo (SP), Brazil; Head of the Dermatology Service at the Pontifícia Universidade Católica de Campinas (PUC-Campinas), Campinas (SP), Brazil; Clinical Director, KOLderma Instituto de Pesquisa Clínica Ltda.—Campinas (SP), Brazil

<sup>2</sup> Dermatology Specialist Candidate at the Dermatology Service of the Pontifícia Universidade Católica de Campinas (PUC-Campinas), Campinas

## Correspondence:

Dr. Adilson Costa  
R. Delfino Cintra, 584—Botafogo  
Cep: 13020-100—Campinas (SP), Brazil  
E-mail: adilson\_costa@hotmail.com

Received on: 9 December 2012

Approved on: 5 March 2013

The present study was carried out at a private practice—Campinas (SP), Brazil.

Financial support: The present study was funded by Mantecorp Indústria Química e Farmacêutica Ltda.—São Paulo (SP), Brazil.  
Conflict of interest: The present study was funded by Mantecorp Indústria Química e Farmacêutica Ltda.—São Paulo (SP), Brazil.

## ABSTRACT

*Hyaluronic acid-based dermal fillers are currently among the most commonly used substances in cosmetic procedures. The present study aims at identifying alterations in the rheological properties of different types of fillers containing hyaluronic acid, before and after flowing through needles. The same analysis is carried out with fillers with and without hyaluronidase.*

**Keywords:** hyaluronic acid; dermis; esthetics.

## RESUMO

Os preenchimentos dérmicos à base de ácido hialurônico constituem procedimentos estéticos dos mais utilizados atualmente. Este trabalho teve como objetivo identificar alterações nas propriedades reológicas dos diferentes tipos de preenchedores com ácido hialurônico antes e após sua passagem pelas respectivas agulhas e, nesta última situação, adicionados ou não de hialuronidase.

**Palavras-chave:** ácido hialurônico; derme; estética.

## INTRODUCTION

Dermal fillers, which are capable of increasing cutaneous volume and restoring facial contours, are an integral part of the armamentarium used in skin rejuvenation.<sup>1,2</sup> Among them, hyaluronic acid (HA) based substances are, without doubt, the most frequently used due to their clinical efficacy and safety of use.

There are currently a number of these filler brands available on the market, which differ in several aspects: purity of raw material, manufacturing process, HA concentration, presence and degree of crosslinking and ability to provide volume and resistance to degradation (enzymes and free radicals).<sup>2</sup> These aspects can play an important role in the behavior of the materials during and after injection.<sup>3</sup>

Rheology is the branch of physics that studies how materials behave in response to forces, when applied to them.<sup>4</sup> Based on such studies, a HA-based filler can be proven to have good rheological stability, remaining intact when passing through a needle or syringe.

Hyaluronidase is an enzyme that acts on the hyaluronic acid present in the extracellular matrix, by breaking the links<sup>1-4</sup> between glucuronic acid and N-acetylglucosamine.

HA-based fillers can be classified into: those that have

crosslinking, those that contain intermolecular bond-generator substances (which increase the stability and durability of the medical implant), and those which do not have crosslinking, i.e. without those stabilizing substances.<sup>6</sup>

There are two types of reticulated HA, which have distinctive characteristics: monophasic and biphasic. The monophasic type consists of a homogeneous blend of high and low molecular weight AHs that are easy to inject and can be sub-classified into monodensified (blending of AHs and reticulation in a single step) or polydensified (cross-linked HA with an increase of crosslinking in a second stage). The biphasic HA are heterogeneous because they contain reticulated HA particles dispersed in the vehicle (non-cross-linked HA) that act as a lubricant, allowing the suspension to pass through a fine needle.<sup>2,7</sup>

A filler's physicochemical structure and rheological properties are important because they can help determine how these substances behave during and after their respective application.<sup>8</sup> Two important rheological properties that can be quantified are: complex viscosity ( $\star$ ) and elastic modulus ( $G'$ ).<sup>8</sup>

During the injection process,  $\star$  refers to the way the filler flows from the needle, i.e. the ability of the fluid phase to resist shear forces, whereas  $G'$  relates to the ability to resist deformation while being injected.<sup>8</sup> Once injected,  $\star$  and  $G'$  influence how the filler resists the skin's tensile forces caused by movement of the face.<sup>8</sup>

A filler with a high  $G'$  value has a greater ability to resist changes in shape.<sup>4</sup> A filler's modulus ( $G'$ ) is influenced by its degree of crosslinking and its concentration – the greater the crosslinking and concentration, the greater the value of  $G'$ .<sup>6</sup> The crosslinking creates intermolecular carbon bridges to hinder the action of endogenous hyaluronidase, which then produces a material with greater longevity and viscoelastic properties.<sup>1,2,6,7</sup>

Fillers with higher concentrations also have a higher volume expansion capacity and may be associated with the increased duration of their effect. Concentrations equal to or greater than 20mg/mL are deemed ideal.<sup>9</sup>

## OBJECTIVE

The present study was aimed at identifying alterations in the rheological properties of different kinds of HA-based dermal fillers before and after their passage through their respective needles and, in the latter situation, when in contact or not with hyaluronidase.

## METHODS

HA-based fillers—and their respective characteristics—used in the present study are shown in Table 1, whose summary is based on information obtained from the currently available literature and advertisements.<sup>7,10,11</sup> Three syringes of each commercially available brand were used. The first (Syringe A) contained the material in its pure state, as found commercially; the second (Syringe B) contained the filler after passing through the needle available in its commercial form, and being returned to its standard syringe; the third (Syringe C) contained the filler after passing through its commercial needle, being returned to its standard syringe through the top brim, and being finally added with 40IU hyaluronidase (Hyalozima® - Apsen Farmacêutica S/A – São Paulo, Brazil).

After being fixed to a flat surface, the syringes were then left upright (at a right angle) for 24 hours. Due to the loss of contents during the replacement of the products to their syringes, and also in consideration of the appropriateness of the photographic images to be obtained, it was decided that only the upper 0.5 mL of each product—measured from the top brim (the tip of the syringe when in the upright, right angle position)—would be evaluated.

After this period, toluidine blue (three drops) was put in all syringes through their tips. The toluidine blue remained in contact with the product at its top. Photographs of the syringes were taken at the time intervals D0 (30' and 2h30'), D1 (24h), D2 (48h), D3 (72h), D4 (96h) and D7 (168h), for macroscopic visualization. At the same time intervals, photographs of the syringes were taken with a Sony CyberShot® DSC-X1 camera

TABLE 1: Types of fillers, concentration, and application site in the dermis

Filler type	Product	Brand	Concentration (mg/mL)	Application site in the dermis	
With crosslinking	Biphasic	Restylane®	Q-Med (Uppsala, Sweden)	20	Middle
		Perfectha Derm®	Obviline (Dardilly, France)	24	Middle
	Monophasic polydensified	Esthelis® Basic	Ateis (Geneva, Switzerland)	22,5	Superficial and middle
Without crosslinking	Monophasic monodensified*	Teosyal® UltradeepLine	Teoxane (Geneva, Switzerland)	25	Middle and deep
		Teosyal® Meso		15	Superficial

Adapted sources: Flynn TC, et al. 2011<sup>7</sup>; Vedamurthy M, et al. 2008<sup>10</sup>; Cornejo P, et al. 2011.<sup>11</sup>

\* Data on file, Teosyal® line (Teoxane Laboratories, Switzerland), obtained from the Brazilian branch

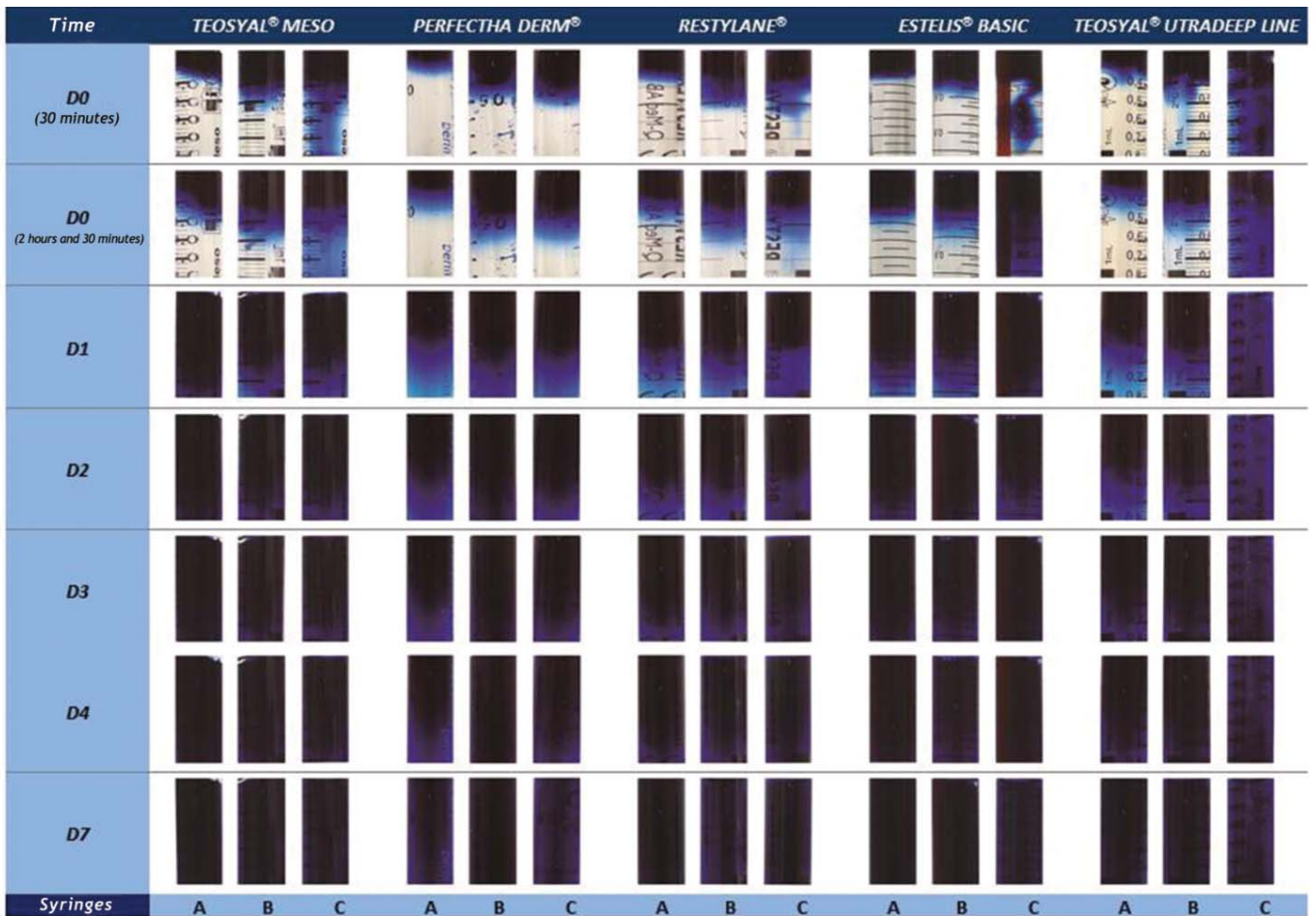


FIGURE 1: Time gravimetry of toluidine blue in different types of HA-based fillers, in different environmental simulations.

(Sony Corporation – Tokyo, Japan) in order to obtain a photographic record of the possible temporal changes.

## RESULTS AND COMMENTS

As can be seen from the images obtained with the evaluation of the toluidine blue's gravimetric migration in the craniocaudal direction of the syringes (Figure 1), it is possible to note that regardless of the HA's concentration and type, the simple fact of the material passing through the needles (Syringe B) alters its state of rheological equilibrium. That situation is accelerated when the products come into contact with hyaluronidase (Syringe C).

In spite of this being an isolated study that evaluated a single syringe of a single specific commercial batch of each product, it is possible to conclude that the products that do not contain crosslinking and are monophasic have an earlier occurrence (D0 – 30') of the bluish stain gravimetry when coming in contact with hyaluronidase (Syringe C). Biphasic products are apparently the ones that have higher rheological stability when in contact with hyaluronidase after passing through the needle. Nevertheless, further studies with a greater number of syringes, obtained from different batches of biphasic and monophasic AH

types, from several brands (especially those who claim to be of the monophasic type, given that they are the most commonly found in the market), should be performed with an aim of determining the actual differences among them, proving the robustness of the findings verified in the present pilot evaluation.

In general, therefore, it was verified that regardless of having crosslinking or not, or a more or less dense HA concentration, the features of HA are influenced by its rheological characteristics due to its passage through the needle, and are intensified when the AH comes into contact with hyaluronidase.

## ACKNOWLEDGEMENTS

We would like to thank the dermatologists Emerson Vasconcelos de Andrade Lima, Márcio Soares Serra, Maria Helena Lesqueves Sandoval and Rosemarie Mazzuco, and the plastic surgeons Henrique Cardoso Tardelli and Henrique Lopes Arantes, who supported the authors by providing scientific information, which allowed the design of this study and the interpretation of the results. ●

**REFERENCES**

1. Brandt FS, Cazzaniga A. Hyaluronic acid gel fillers in the management of facial aging. *Clin Interv Aging*. 2008;3(1):153-9.
2. Monteiro EO, Parada MOB. Preenchimentos faciais: parte um. *RBM - Rev Bras Med (Especial Dermatologia)*. 2010;67(7):6-14.
3. Smith KC. Reversible vs. nonreversible fillers in facial aesthetics: Concerns and considerations. *Dermatol Online J*. 2008;14(8):3.
4. Stocks D, Sundaram H, Michaels J, Durrani MJ, Wortzman MS, Nelson DB. Rheological Evaluation of the Physical Properties of Hyaluronic Acid Dermal Fillers. *J Drugs Dermatol*. 2011;10(9):974-980.
5. Hynes WL, Walton SL. Hyaluronidase of gram-positive bacteria. *FEMS Microbiol Lett*. 2000;183(2):201-7.
6. Falcone RA, Berg SJ. Crosslinked hyaluronic acid dermal fillers: a comparison of rheological properties. *J Biomed Mater Res A*. 2008;87(1):264-71.
7. Flynn TC, Sarazin D, Bezzola A, Terrani C, Micheels P. Comparative Histology of Intra-dermal Implantation of Mono and Biphasic Hyaluronic Acid Fillers. *Dermatol Surg*. 2011;37(5):637-43.
8. Sundaram H, Voigts B, Beer K, Meland M. Comparison of the Rheological Properties of Viscosity and Elasticity in Two Categories of Soft Tissue Fillers: Calcium Hydroxylapatite and Hyaluronic Acid. *Dermatol Surg*. 2010;36(Suppl 3):1859-65.
9. Gold MH. Use of Hyaluronic Acid Fillers for the Treatment of the aging face. *Clin Interv Aging*. 2007;2(3):369-76.
10. Vedamurthy M, Vedamurthy A. Dermal fillers: tips to achieve successful outcomes. *J Cutan Aesthet Surg*. 2008;1(2):64-7.
11. Cornejo P, Alcolea JM, Trelles MA. Perspectivas en el uso de materiales de relleno inyectables para tejidos blandos, desde nuestra experiencia. 1ª Parte. *Cir Plast Iberolatinoam*. 2011;37(4):393-402.