In vitro evaluation of viscosity of facial hyaluronic acid fillers through shear tension analysis. Preliminary data

Avaliação *in vitro* da viscosidade de preenchimentos faciais de ácido hialurônico através da análise de tensão de cisalhamento. Dados preliminares

Evaluación *in vitro* de la viscosidad de los rellenos faciales de ácido hialurónico mediante análisis de tensión de cizallamiento. Datos preliminaries

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Abstract
To analyze and describe the viscosity and the stress in relation to the shear rate of eleven facial fillers of four commercial HA brands with authorized use in Brazil through the flow sweep rheological analysis method. The rheological parameter of viscosity (η) was analyzed by rheometry tests through flow sweep. The behavior of shear stress showed that Rennova Fill Lido® and Lift Lido® have higher viscosity ratios (η = 336 Pa.s and η = 406 Pa.s, respectively). In contrast, Hialurox® brand fillers showed the lowest viscosity value (η) (Hialurox Ultra Soft 8 mg/g® η= 9 Pa.s, Fine 16 mg/g η= 29 Pa.s, Fill 24 mg/g η= 36 Pa.s, Lift 26 mg/g η= 50 Pa.s). The flow sweep showed variations in the viscosity (η) of the eleven HA dermal fillers. Rennova Fill Lido® and Lift Lido® have a lower degree of spreadability and are less susceptible to shear forces and yield strength, making them ideal candidates for the intended treatment.
Keywords: Hyaluronic acid; Fillers; Rheological properties; Rejuvenation; Viscosity.

Resumo
Analisar e descrever a viscosidade e a tensão em relação à taxa de cisalhamento de onze preenchedores faciais de quatro marcas comerciais de HA com uso autorizado no Brasil por meio do método de análise reológica de varredura de fluxo. O parâmetro reológico de viscosidade (η) foi analisado por ensaios de reometria por varredura de fluxo. O

comportamento da tensão de cisalhamento mostrou que Rennova Fill Lido® e Lift Lido® apresentam maiores razões de viscosidade (η = 336 Pa.s e η = 406 Pa.s, respectivamente). Em contraste, os fillers da marca Hialurox® apresentaram o menor valor de viscosidade (η) (Hialurox Ultra Soft 8 mg/g® η= 9 Pa.s, Fine 16 mg/g η= 29 Pa.s, Fill 24 mg/g η= 36 Pa.s, Lift 26 mg/g η= 50 Pa.s). A varredura de fluxo mostrou variações na viscosidade (η) dos onze preenchedores dérmicos de HA. Rennova Fill Lido® e Lift Lido® têm um menor grau de espalhabilidade e são menos suscetíveis a forças de cisalhamento e resistência ao escoamento, tornando-os candidatos ideais para o tratamento pretendido.
Palavras-chave: Ácido hialurônico; Preenchedores; Propriedades reológicas; Rejuvenescimento; Viscosidade.

Resumen

Analizar y describir la viscosidad y el estrés en relación con la velocidad de corte de once rellenos faciales de cuatro marcas comerciales de HA con uso autorizado en Brasil a través del método de análisis reológico de barrido de flujo. El parámetro reológico de viscosidad (η) se analizó mediante ensayos de reometría mediante barrido de flujo. El comportamiento del esfuerzo cortante mostró que Rennova Fill Lido® y Lift Lido® tienen relaciones de viscosidad más altas (η = 336 Pa.s y η = 406 Pa.s, respectivamente). Por el contrario, los rellenos de la marca Hialurox® mostraron el valor de viscosidad más bajo (η) (Hialurox Ultra Soft 8 mg/g® η= 9 Pa.s, Fine 16 mg/g η= 29 Pa.s, Fill 24 mg/g η= 36 Pa.s, Lift 26 mg/g η= 50 Pa.s). El barrido de flujo mostró variaciones en la viscosidad (η) de los once rellenos dérmicos de HA. Rennova Fill Lido® y Lift Lido® tienen un menor grado de capacidad de esparcimiento y son menos susceptibles a las fuerzas de cizallamiento y el límite elástico, lo que los convierte en candidatos ideales para el tratamiento previsto.
Palabras clave: Ácido hialurónico; Rellenos; Propiedades reológicas; Rejuvenecimiento; Viscosidad.

1. Introduction

Aging is responsible for changing the harmonious and symmetrical features found in the face during youth. These changes are unfavorable and result in a less attractive facial appearance, with consequences both in physical attractiveness and decreased self-esteem (Furtado et al., 2023; Swift et al., 2021).

The first signs of facial aging are reflected in the appearance of wrinkles and folds, accompanied by changes in skin color and texture. Besides promoting an unbalanced appearance of soft tissues, these associated factors cause changes in self-perception and can lead to psychological, emotional, and social problems (Reilly et al., 2015).

Aging evokes essential changes in the craniofacial skeletal set that structure the soft tissues, causing their stability and definition. As age advances, skeletal density decreases, initiating a bone remodeling event that leads to ptosis of the adjoining soft tissues. When bone remodeling happens in the middle third of the face, disharmonies in the upper, middle and lower thirds of the face are clearly noticeable (Kahn & Shaw, 2010; Truswell, 2013; Swift et al., 2021).

It is known that bone remodeling also influences facial fat compartments, which are often repositioned in the periorbital region, the medial portion of the face, and the mandible. This phenomenon occurs because the fat pads are moved by bone changes and also due to the weakening of the supporting ligaments (Farkas et al., 2013; Wulc et al., 2012), leading to deepening of the temples (Foissac et al., 2017) and inferomedial displacement of the skin (Wulc et al., 2012). This favors the appearance of the characteristic rhytides and furrows of the aging face.

Muscles are also part of the complex structures that suffer from the senescence effect related to aging. Changes in muscle fiber composition and, consequently, in the tone of the muscles of the mimic through repetitive muscle contraction influence the suspension and structural integrity of the soft tissues, modifying the volume and facial contour (Papageorgiou et al., 2012; Yun et al., 2014).

Current facial aesthetic treatments seek to mitigate the signs of aging and achieve results closer to a youthful face with harmonious, symmetrical, and balanced features (Swift et al., 2021). To achieve these effects, Hyaluronic Acid (HA) facial fillers have been widely used to correct volume loss and treat facial wrinkles (Ho & Jagdeo, 2015; Billon et al., 2016). However, depending on the application technique and the rheological characteristic of the product, the HA may be subjected to lateral shear and compression forces, resulting in over- or under-correction of the desired facial contour and failure of the proposed treatment (Billon et al., 2016).

One of the critical parameters to verify the performance of HA in facial soft tissues is through its physical-chemical characterization based on rheological analysis to determine its viscoelastic property under shear stress (Molliard et al., 2018).

However, in the literature searched, no studies were found that objectively described this property of HA, particularly those approved by the Brazilian Health Surveillance Agency (ANVISA) and used in Brazil. Thus, the purpose of this study was to analyze and describe the viscosity and the stress in relation to the shear rate of eleven facial fillers of four commercial HA brands with authorized use in Brazil through the flow sweep rheological analysis method.

2. Methodology

2.1 Materials

Eleven fillers of four HA brands intended for facial aesthetic treatments in Brazil were submitted to flow sweep in order to analyze and describe the viscosity and stress in relation to the shear rate. A Rheometer (TA-Instruments AR-1500ex®, New Castle - DE, England) was used. The eleven HA characterized belonged to the brands e.p.t.q® (S100, S300, S500; Jetema Co., Ltd, South Korea), Hialurox® (Ultra Soft 8mg/g®, Ultra Fine 16mg/g®, Ultra Fill 24mg/g®, Ultra Lift 26mg/g®; Hialurox® Ltda, Sao Carlos, Brazil), Perfecta® (Derm Subskin®; Sinclair Pharma, France), Rennova® (Fill Lift®; InnovapharmA/Croma-Pharma, Austria), which are described in Table 1.

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Table 1 - Identification and clinical indication of the dermal fillers used in the study.

Brand	Batch	Manufacturer's clinical indication
e.p.t.q S 100®	YLA20007	Superficial Dermis - Frontal Region, temple, lower eyelid
e.p.t.q S 300®	YLB21002	Medium and deep dermis - Frontal region, glabella, nasolabial fold, upper lip contour, and filter
e.p.t.q S 500®	YLC21002	Deep subcutaneous - malar region, nasal dorsum, tip of the nose, columella, lips, chin
Hialurox Ultra Soft 8 mg/g®	OFH-SOFT 210810-01	More superficial static wrinkles - periorbital region, soft lip contour, and fillers
Hialurox Ultra Fine 16 mg/g®	OFH-FINE 210720-01	Less deep static wrinkles - Lip contour and fillers
Hialurox Ultra Fill 24 mg/g®	OFH-FILL 210713-01	Deep static wrinkles - Malar region, nasolabial fold, labiomental fold, rhinomodeling, chin modeling
Hialurox Ultra Lift 26 mg/g®	OFH-LIFT 210608-01	Malar, nasolabial fold, labiomental fold, rhinomodeling, chin modeling
Perfectha Derm®	200928-2	Subcutaneous injection - Medium lines - Nasolabial folds, marionette lines, lip enhancement, and scars
Perfectha Subskin®	201109-1	Deep subcutaneous and supraperiosteal injection - facial atrophy, redefine contours, add volume to cheeks, chin, temples, forehead, nose tip
Rennova Fill Lido®	105043	Mid to subdermal dermis - perioral lines, nasojugal lines, periorbital lines, lip volume and contour, light marionette lines, and fine wrinkles
Rennova Lift Lido®	(10) 204452	Subdermal and superficial subcutaneous - Nasolabial fold, chin, marionette lines, malar region, temple, oral commissure

Source: Authors (2023).

2.2 Flow sweep test

The flow sweep test allows viscosity and stress to be measured in relation to the shear rate. The samples were tested using 0.9 mL of each filler at a temperature of 25 °C under a shear rate of 0.01 to 100 s^{-1} with a parallel-plates geometry, 40 mm sandblasted, and a 500 µm gap between the parallel plate and the rheometer to obtain the viscosity measurements. The viscosity value (η) was measured at the shear rate at $1s^{-1}$ (Molliard et al., 2018).

2.3 Analysis of the collected data

All results were assessed by comparing the viscosity values with each other and treated by descriptive statistics.

3. Results

The comparative viscosity (η) results measured by the flow sweep test in shear stress of the eleven HA fillers are summarized in Table 2.

Table 2 - Viscosity (η) of the samples analyzed in this study at 1s-1 shear rate.

Analysis	Sample Name	Viscosity (η) at $1s^{-1}$ (Pa.s)
1	e.p.t.q S 100®	130
2	e.p.t.q S 300®	228
3	e.p.t.q S 500®	286
4	Hialurox Ultra Soft 8 mg/g®	9
5	Hialurox Ultra Fine 16 mg/g®	29
6	Hialurox Ultra Fill 24 mg/g®	36
7	Hialurox Ultra Lift 26 mg/g®	50
8	Perfectha Derm®	202
9	Perfectha Subskin®	189
10	Rennova Fill Lido®	336
11	Rennova Lift Lido®	403

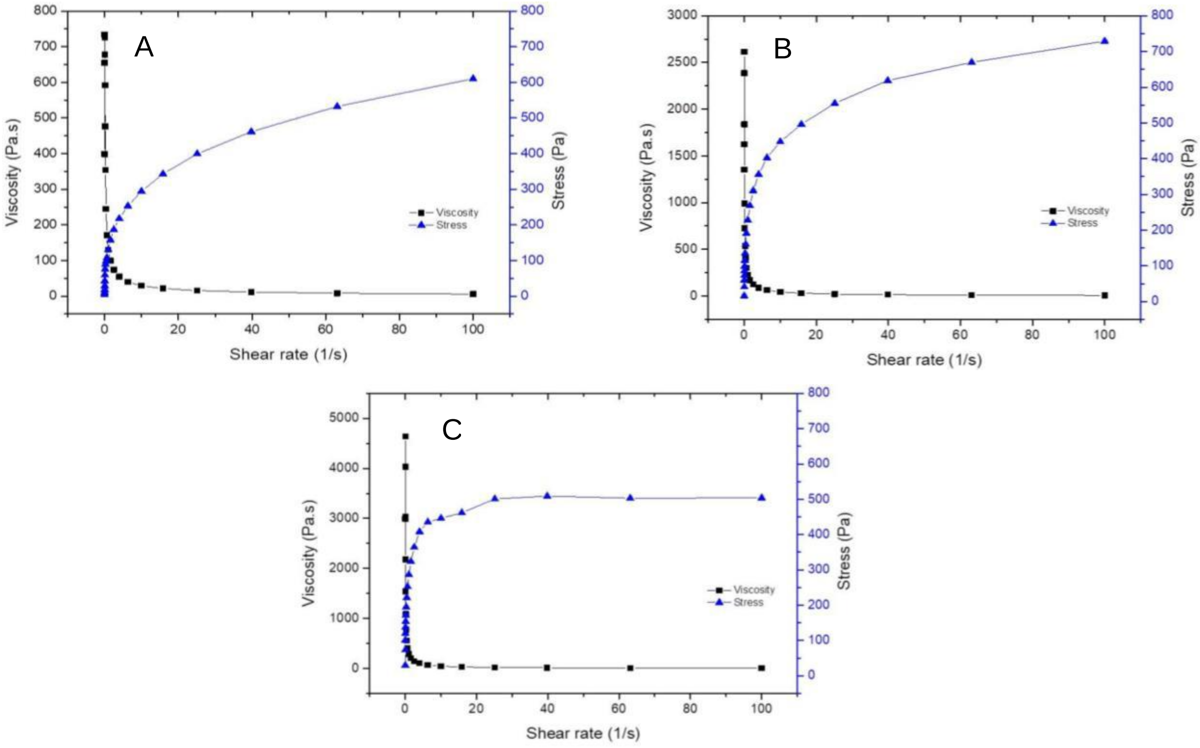
Source: Authors (2013).

The behavior of shear stress in relation to viscosity (η) applied to the fillers of four brands marketed in Brazil (Table 2) showed that Rennova Fill Lido® and Lift Lido® hold the highest viscosity rate among all the HA gels investigated in this study (η = 336 and 403 Pa.s), respectively). Furthermore, it is observed that the HA e.p.t.q S300® and S500® (η = 228 and 286 Pa.s, respectively), as well as Perfecta Derm® (η = 202 Pa.s) and Subskin® (η = 189 Pa.s) revealed close viscosity values (Table 2).

All Hyalurox® brand fillers reported minimal viscosity values when compared to the other samples (Table 2). That is, its viscosity is apparently affected by shear stress. The e.p.t.q S100® showed intermediate viscosity (η = 130 Pa.s) compared to the materials with high viscosity rate and even the fillers from its own commercial line.

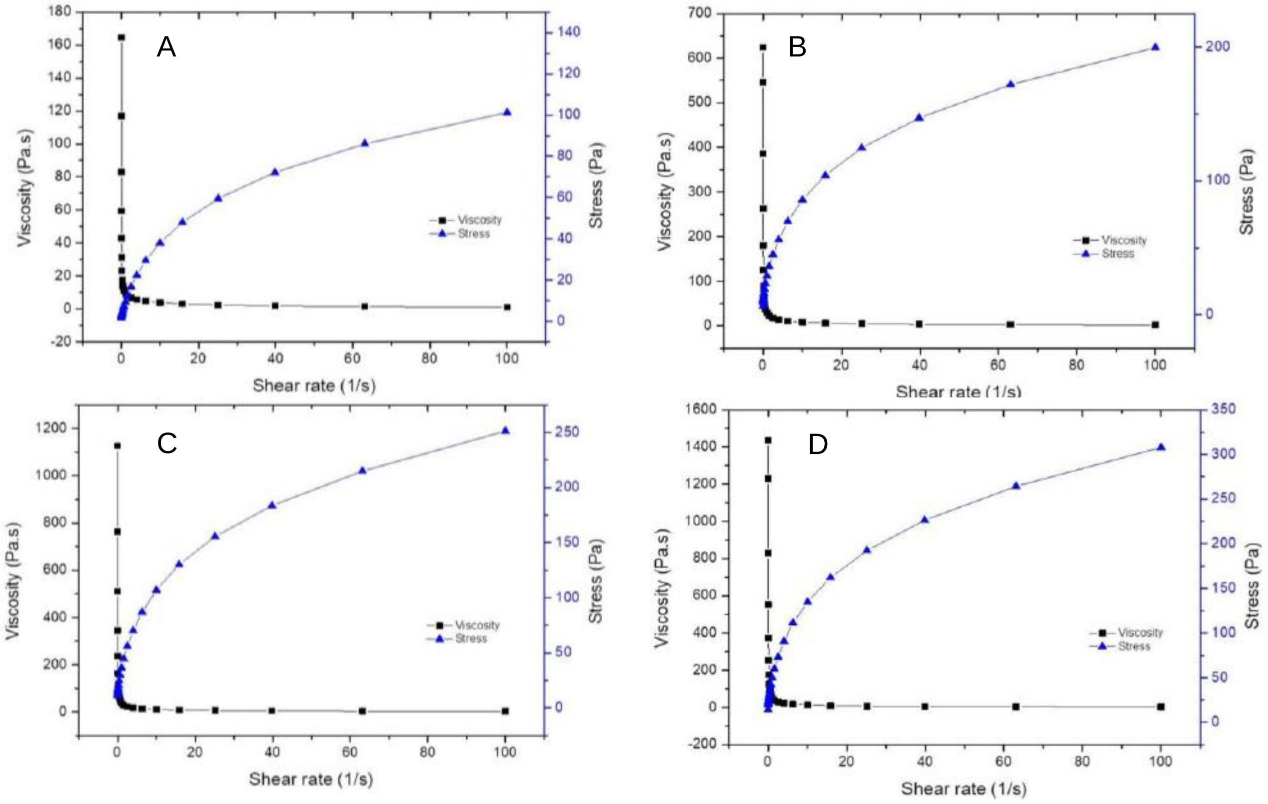
All curves in the analysis showed pseudoplastic flow behavior with thixotropic characteristics. This behavior is characterized by the decrease in viscosity with the increase in shear rate, as can be seen for the e.p.t.q® (Figure 1A-C), Hialurox® (Figure 2A-D), Perfecta® (Figure 3A-B) and Rennova® (Figure 4A-B) HA fillers.

Figure 1 - Viscosity (black) and flow (blue) curves for S100® (A), S300 (B), and S500 (C) e. p. t. q fillers.



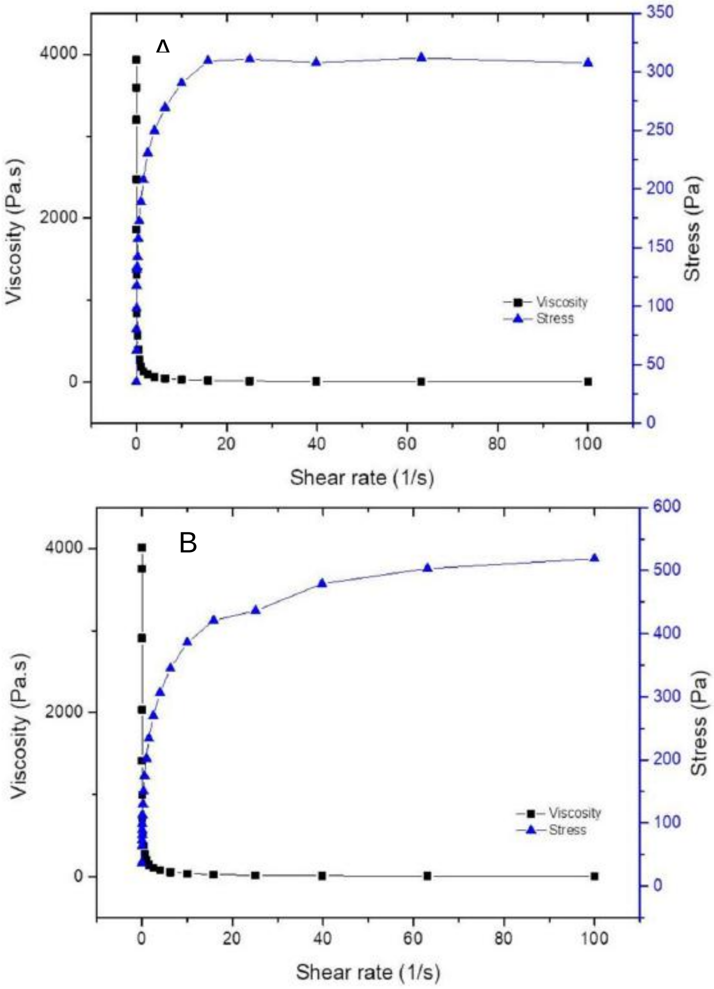
Source: Authors (2013).

Figure 2 - Viscosity (black) and flow (blue) curves for Hyalurox® fillers. Hialurox Ultra Soft 8mg/g® (A), Hialurox Ultra Fine 16 mg/g® (B), Hialurox Ultra Fill 24 mg/g® (C), Hialurox Ultra Lift 26 mg/g® (D).



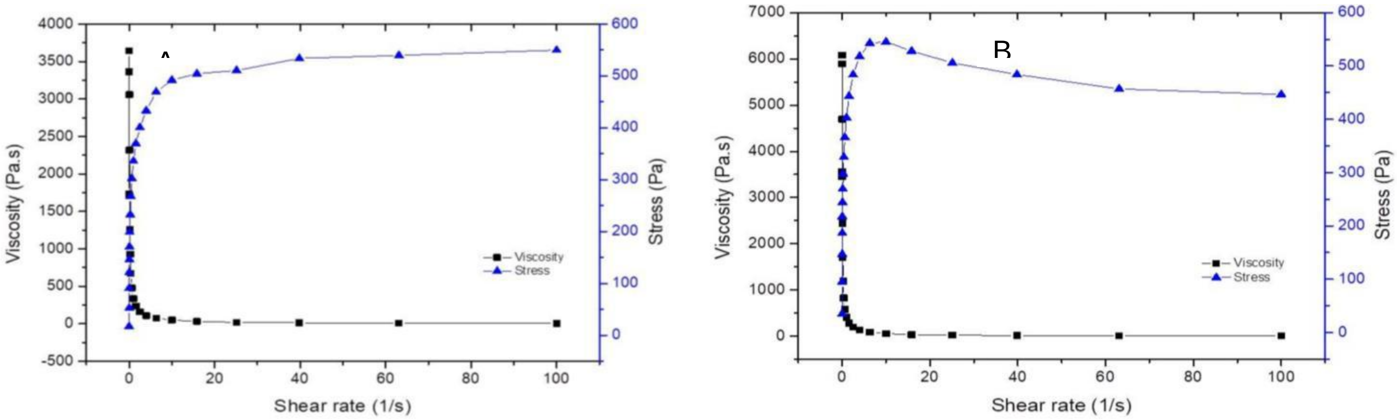
Source: Authors (2013).

Figure 3 - Viscosity (black) and flow (blue) curves for Perfecta® fillers. Perfecta Subskin® (A), Perfecta Derm® (B).



Source: Authors (2013).

Figure 4 - Viscosity (black) and flow (blue) curves for Rennova®. Rennova Fill Lido® (A), Perfecta Lift Lido® (B).



Source: Authors (2013).

The figures show that the viscosity is one order of magnitude than dependent of the type materials used in the essay and that these differences may be the target of selecting these materials in the clinical stage.

4. Discussion

The set of structures such as bones, muscles, fat compartments, blood vessels, and skin present on the face evidence that this human body region is complex and highly dynamic. In aesthetic medicine, facial rejuvenation treatments must be approached with a set of safe techniques. The use of fillers must be well evaluated based on their adequate indication after diagnosis since these materials suffer variations according to the lateral shear and compression rates of the anatomical plane that will receive them (Billion et al., 2016).

All fillers evaluated in this study had their viscosity reduced as shear velocity and stress increased (Figures 1-4). This shows that the molecules of the HA formulation are oriented in the direction of the applied force. The more viscous fillers presumably exhibited lower shear stress when implanted into the face's soft tissues, exhibiting pseudoplastic flow (Pisárčik et al., 1995). Furthermore, the analysis curves of the fillers (Figures 1-4) show that the HA formulations did not undergo drastic changes in shear, suggesting that these gels maintained their structure closer to the natural one.

The ability to resist deformation of HA gels with high viscosity (Rennova Fill Lido® and Lift Lido®), seen in Table 2, is essential to consider during and after esthetic treatment. In addition, more viscous injectable HA tends not to move easily toward lower strength, achieving desirable correction in smaller extensions (Molliard et al., 2018). These indications are shown in Figures 1-4 for the viscosity of the fillers analyzed in this study, where less viscous fillers may spread more easily and make it more difficult to achieve modeling of the region to which volume recovery is proposed.

As shown in Table 2 and Figure 4A-B, Rennova® fillers showed higher viscosity coefficient, which may mean that both are able to resist the flow when under tension force in the facial soft tissues. Furthermore, the behavior of all samples was non-linear, as there was variation in the viscosity path under shear stress.

However, increasing the viscosity of a given fluid within connective tissue can decrease its glide during muscle movements (Stecco et al., 2013). When this phenomenon applies to facial mimic movements, increased product stiffness in the implanted plane may occur, resulting in a less attractive aesthetic appearance. For this reason, higher viscosity HA fillers may have a better indication in these cases because these need to be injected in deeper layers and with greater extrusion force in order to control this phenomenon.

Additionally, when considering volumizing in an aesthetic facial harmonization treatment, the practitioner should apply the product at the deep dermis level. When the product is injected in the subcutaneous layer, there is a risk of vascular compression and/or vascular occlusion (Lee et al., 2018).

When viscosity and cohesiveness characteristics are added to the fillers, they increase their ability to mold after injection during the accommodation massage (Molliard et al., 2018). Thus, the union of these parameters allows the product to be adequately injected, distributed, and homogenized in the tissues without fragmentation of the material (Molliard et al., 2018).

Table 2 shows the viscosity results (η) for each dermal filler brand. As such, it can be seen that the product's viscosity will depend mainly on the concentration of HA in its formulation, and this behavior was clearly identified in the four Hyalurox® HA presentations, where low viscosity is indicated for the treatment of fine lines (Molliard et al., 2018). For these reasons, the indications, when described by the manufacturers, should be strictly observed to avoid unpredictable results and undercorrections. The practitioner needs to understand the indications of the products and the material's properties. Thus, understanding the properties evaluated in this study will give confidence to the professional when deciding which material to use based on the clinical diagnosis.

There is a certain lack of knowledge on the part of those who use the material, which may compromise the desired treatment results. Additionally, the recommendation of the injection plane is usually made based on the Elastic modulus (G') (Fagien et al., 2019. In addition to viscosity, other physicochemical properties are also involved in the performance of HA gels, such as crosslinking reactions, molecular weight, concentration, and the process by which the HA is fragmented. These will provide the mechanical basis for the strength and longevity of the product (Kenne et al., 2013; Lorenc et al., 2013; Borzacchiello et al., 2015. All of these properties need to be considered when selecting the material at the time of use.

A characteristic example is related to the HA's water retention capacity, which is directly associated with the degree of crosslinking of the filler. Thus, the crosslinking reactions are formulated according to the intended use, where a low crosslinking filler is indicated for superficial planes, while products with higher crosslinking are intended for deeper planes (Kablik et al., 2009; Sundaram et al., 2010).

The repositioning of facial tissues to treat ptosis is one of the primary goals of facial harmonization, and this can be achieved by planning with HA injecting it into strategic regions of the face. Therefore, the choice of the appropriate material is an unquestionable option because the lifting capacity of the dermal filler is associated with the strength and viscosity of the gel itself (Borrell et al., 2011; Edsman et al., 2012).

In addition to replenishing the soft tissues of the face, the fillers with greater volumizing capacity have indications of restoring volume to the cheeks, malar, chin, temples, and jaws, allowing optimizing the aesthetic results and the satisfaction of patients who present such facial irregularities (Betemps et al., 2018).

Among the materials studied, the one that presented adequacy for deep applications was the Hialurox® brand, where the values obtained and presented in Table 2 and Figures 2C-D demonstrate that, for the purposes of this study, results for filling deeper regions can be obtained by fillers with the characteristics Hialurox Ultra Fill 24 mg/g® and Hialurox Ultra Lift 26 mg/g® (η = 36 Pa.s and η = 50 Pa.s, respectively). However, other properties not investigated in this experiment should be considered. This is because a low viscosity HA seems to exhibit greater fluidity and spread in tissues and is, therefore, more modifiable after its injection during the massage, resulting in a better distribution in tissues with little fragmentation of the product (Molliard et al., 2018). It would allow us to assume less nodule formation as a characteristic of postoperative reaction (Micheels et al., 2013). This evidence can only be confirmed in properly randomized and controlled clinical trials, and this experiment cannot conclude that these clinical facts really happen since only one of the properties of hyaluronic acids was investigated.

It is worth noting that dermal quality is an essential factor in directing the most appropriate selection of HA. In such a way, the degree of skin laxity and resistance will lead to the necessary and individual correction for each patient, designating the choice of filler according to its strength, firmness, and distribution (Fagien et al., 2019).

In facial planning, it is estimated that the result of the aesthetic treatment projects the tissues and that the HA gel is resistant to deformation from the shear forces it will suffer throughout its longevity after implantation (Edsman et al., 2012). As long as the gel does not undergo total degradation, it will continue correcting expression lines and wrinkles and restoring volume lost by the senescence process (Edsman et al., 2012).

Cosmiatry has been concerned with developing products with low viscosity and high shear rate to flow without difficulty during extrusion through a needle or cannula. Thus, the lower the viscosity at a high shear rate, the lower the chances of the filler deforming because the professional will exert less extrusion force on it (Molliard et al., 2012). Viscosity rates should be considered when choosing the product, especially when dealing with highly mobile areas and treatments requiring large amounts of product. In addition, these differences can be seen as a result of the different manufacturing processes of each brand, which presupposes a specific indication according to the product's characteristics.

Since this is an unprecedented and non-comparative experiment over time, this experiment achieves its main proposal, which is to expose, through flow sweep test, the behavior of the different materials approved by ANVISA and marketed in Brazil through the exposure of preliminary data of these investigated products. However, more information is needed for the conclusions about the consistency of their indications. Therefore, it is suggested that controlled and randomized clinical trials be conducted so that accurate conclusions can be attributed to these different materials.

5. Conclusion

The flow sweep showed variations in the viscosity (η) of the eleven HA dermal fillers showing that Rennova Fill Lido® and Lift Lido® have a lower degree of spreadability and are less susceptible to shear forces and yield strength, making them ideal candidates for the intended treatment. In this regard, the preliminary data from this pilot study strongly indicate that it is necessary to understand the rheological properties of HA-based facial dermal fillers in order to understand that shear force may influence facial filler results, analogous to the force exerted during product injection. Therefore, new studies are still needed to establish a correlation between in vitro rheometric analyzes with the performance of in vitro fillers alive.

References

Betemps, J. B., Marchetti, F., Lim, T., Hadjab, B., Micheels, P., Salomon, D., & Molliard, S. G. (2018). Projection capacity assessment of hyaluronic acid fillers. *Plastic and Aesthetic Research*. 5, 19.

Billon, R., Hersant, B., & Meningaud, J. P. (2016). Rhéologie des acides hyaluroniques: principes fondamentaux et applications cliniques en rajeunissement facial. *Annalles de Chirurgie Plastiques Esthétique*. 62(3), 261-267.

Borrell, M., Leslie, D. B., & Tezel, A. (2011). Lift capabilities of hyaluronic acid fillers. *Journal of Cosmetic and Laser Therapy*. 13(1), 21-27.

Borzacchiello, A., Russo, L., Malle, B. M., Schwach-Abdellaoui, K., & Ambrosio, L. (2015). Hyaluronic acid based hydrogels for regenerative medicine applications. *Biomed Research International*. 2015, 871218.

Edsman, K., Nord, L. I., Ohrlund, A., Larkner, H., & Kenne, A. H. (2012). Gel Properties of Hyaluronic Acid Dermal Fillers. *Dermatologic Surgery*. 38(7), 1170-1179.

Farkas, J. P., Pessa, J. E., Hubbard, B., & Rohrich, R. J. (2013). The science and theory behind facial aging. *Plastic Reconstructive Surgery Global Open*. 1(1), e8-e15.

Foissac, R., Camuzard, O., Piereschi, S., Staccini, P., Andreani, O., Georgiou, C., & Benatar, M. (2017). High-Resolution Magnetic Resonance Imaging of Aging Upper Face Fat Compartments. *Plastic and Reconstructive Surgery*. 139(4), 829-837.

Furtado, G. R. S., Barbosa, K. L., Dametto, A. C., Sisnando, A. L., da Silva, L. C. F., Souza, J. R. A., de Oliveira, R. C. G., Albergaria-Barbosa, J. R., & Rizzatti-Barbosa, C. M. (2023). Rheological properties of hyaluronic acid-based fillers for facial cosmetic aesthetics. *Research, Society and Development*. 12(1), e22012139676.

Ho, D., & Jagdeo, J., Biological properties of a new volumizing hyaluronic acid filler: a systematic review. *Journal of Drugs in Dermatology*. 14(1), 50-54.

Kablik, J., Monheit, G. D., Yu, L., Chang, G., & Gershkovich, J. (2009). Comparative physical properties of hyaluronic acid dermal fillers. Dermatologic Surgery. 35(1), 302-312.

Kahn, D. M., & Shaw, R. B. (2010). Overview of current thoughts on facial volume and aging. *Facial Plastic Surgery*. 26(5), 350-355.

Kenne, L., Gohil, S., Nilsson, E. M., Karlsson, A., Ericsson, D., Kenne, A. H., & Nord, L. I. (2013). Modification and crosslinking parameters in hyaluronic acid hydrogels: Definitions and analytical methods. *Carbohydrate Polymers*. 91(1), 410-418.

Lee, W., Yoon, J. H., Koh, I. S., Oh, W., Kim, K. W., & Yang, E. J. (2018). Clinical application of a new hyaluronic acid filler based on its rheological properties and the anatomical site of injection. *Biomedical Dermatology*. 2(22), 1-5

Lorenc, Z. P., Fagien, S., Flynn, T. C., & Waldorf, H. A. (2013). Clinical application and assessment of Belotero: A roundtable discussion. Plastic and Reconstructive Surgery. 132(4), 69S-76S.

Micheels, P., Sarazin, D., Besse, S., Sundaram, H., & Flynn, T. C. (2013). A blanching technique for intradermal injection of the hyaluronic acid Bolotero. *Plastic and Reconstructive Surgery*. 132(4), 59S-68S.

Molliard, S. G., Bétemps, J. B., Hadjab, B., Topchian, D., Micheels, P., & Salomon, D., (2018). Key rheological properties of hyaluronic acid fillers: from tissue integration to product degradation. *Plastic and Aesthetic Research*. 5:17.

Papageorgiou, K. I., Mancini, R., Garneau, H.C., Chang, S. H., Jarullazada, I., King, A., Forster-Perlini, E., Hwang, C., Douglas, R., & Goldberg, R. A. (2012). A three-dimensional construct of the aging eyebrow: the illusion of volume loss. *Aesthetic Surgery Journal*. 32(1), 46-57.

Pisárčik, M., Bakoš, D., & Čeppan, M. (1995). Non-Newtonian properties of hyaluronic acid aqueous solution. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 97(3), 197-202.

Reilly, M. J., Tomsic, J. A., Fernandez, S. J., & Davison, S. P. (2015). Effect of facial rejuvenation surgery on perceived attractiveness, femininity, and personality. *JAMA Facial Plastic Surgery*. 2015; 17(3), 202-207.

Stecco, A., Gesi, M., Stecco, C., & Stern, R. (2013). Fascial components of the myofascial pain syndrome. Current Pain and Headache Reports. 17(8), 352.

Sundaram, H., Voigts, B., Beer, K., & Meland, M. (2010). Comparison of the rheological properties of viscosity and elasticity in two categories of soft tissue fillers: Calcium hydroxylapatite and hyaluronic acid. *Dermatologic Surgery*. 36(3), 1859-1865.

Swift, A., Liew, S., Weinkle, S., Garcia, J. K., & Silberberg, M. B. (2021). The Facial Aging Process From the “Inside Out”. *Aesthetic Surgery Journal*.

Truswell, W. H. T. (2013). Aging changes of the periorbita, cheeks, and midface. *Facial Plastic Surgery*. 2013; 29(1), 3-12.

Wulc, A. E., Sharma, P., & Czyz, C. N. (2012). The anatomic basis of midfacial aging. In: Hartstein, M. E., Wulc, A. E., Holck, D. E., eds. Midfacial Rejuvenation. New York, NY: Springer New York; 15-2.

Yun, S., Son, D., Yeo, H., Kim, S., Kim, J., Han, K., Lee, S., & Lee, J. (2014). Changes of eyebrow muscle activity with aging: functional analysis revealed by electromyography. *Plastic and Reconstructive Surgery*. 133(4), 455e-463e