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ORIGINAL ARTICLE

Rheological properties of cross-linked hyaluronic acid dermal fillers

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ABSTRACT
Aim: **Hyaluronic acid (HA) based dermal fillers (DF) in recent years aroused big interest in the area of cosmetic surgery for the rejuvenation of the dermis. There is not a ideal dermal filler for all applications and in commerce there are many types of DF that differ for their chemical-physical properties. The aim of this paper is to correlate the rheological and physical properties of different DF to their clinical effectiveness.**
Matherials and Methods: **In this frame the samples have been subjected to oscillation dynamic rheological and steady shear measurements.**
Results: **Our results demonstrate that the viscoelastic properties of different DF varie strongly also considering fillers of the same family. Furthermore it was found that the materials physical properties influence significantly the performance of dermal filler. In particular the clinical data appear to correlate with the concentration of the polymer and with the product between the concentration and the percent elasticity, so these should be crucial parameters for the clinical performance of DF.**
Conclusion: **Rheological data can be a tool to have an indication on the efficacy and longevity of DF but it has to be considered that production technology, in-vivo-conditions, injector skills and experience also influence them significantly.**

Key words: Dermal fillers, Hyaluronic acid, Hydrogels, Viscoelastic properties, Viscosity

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INTRODUCTION

Injectable dermal fillers (DF) represent a real aesthetic revolution because they have become important alternatives to traditional surgical procedures to correct facial wrinkles and to modify the volume and appearance of the lips. The goal of these materials is to restore a youthful appearance by augmenting the soft tissue; in fact, the concentration of important structural components of the dermis, such as collagen and hyaluronic acid, decreases with age, promoting the formation of wrinkles formation (1). With the use of dermal fillers it is possible to replace them, achieving a natural and younger appearance.

During the early 1990s, the use of paraffin or silicone as a filler became popular (2). Then the substance used for soft tissue augmentation was injectable bovine collagen, but this, after the injection, was recognized by the immune system as a foreign substance and its use was discontinued in a few months. Furthermore, bovine collagen, when used as a dermal filler, raised hypersensitivity and inflammatory reactions. The last generation of dermal fillers is hyaluronic acid (HA) based. HA is a naturally occurring linear polysaccharide composed of repeating disaccharide units of D-glucoronic acid and N-acetyl glucosamine linked by β 1-3 and β 1-4 glucosidic bonds (3-7) (Fig. 1). HA is a primary component of the extracellular matrix of the mammalian connective tissues and it is an important structural element in the skin; HA is present in high concentration in the synovial joint fluids, vitreous humor of the eyes, hyaline cartilage, disc nucleus and umbilical cord (8-12). HA plays a major role in several functions in vivo such as lubrification of arthritis joints, viscoelastic properties of soft tissue and it is involved in important cell functions such as cell motility, cell matrix adhesion and cell organization (13-17). Thanks to its physical, chemical and biological properties HA has generated increasing interest among researchers and it is already used in several biomedical applications (18,19).

HA and its derivatives, such as cross-linked HA materials (CLHA), are particularly suitable for soft tissue augmentation since they can be injected superficially because they are colorless. Moreover, an allergic skin test is unnecessary because chemical structure of HA is uniform in all living species, so the probability of immunogenicity is low (20). Nevertheless, the correction is not permanent, because the hyaluronic acid is degraded over time and the injection site re-treatments should be taken into account (21).

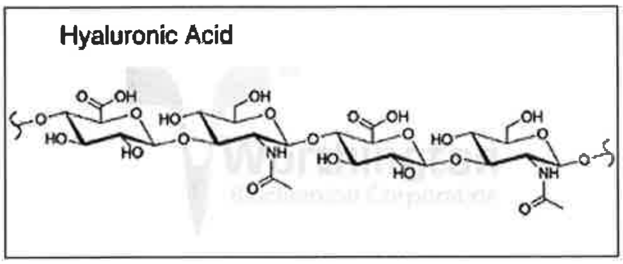

Fig. 1 - *Chemical structure of HA.*

There are several kinds of HA-based DF in commerce, but there is no ideal DF for all indications. There are many factors, i.e. the physical and chemical properties of DF, that can significantly influence the persistence and desired properties of DF for the particular application. For example, particle size influences the degree of correction and volume filling, polymer concentration is one of the factors that influences the longevity and stability of filler, the swelling of gel is related to the degree of inflammation and induration (22). Furthermore, the performance of DF can depend on the density of cross-linking agents, viscosity, extrusion force, stress applied, etc. For example, DF, during their use, are subjected to high and low stress; in fact, a high stress is applied when it is injected through the small bore needle, so in this case DF must have a low viscosity; instead, when the materials are at the site of application, there is a low stress environment, and the DF must be predominately elastic (23).

The number of DF commercially available has increased significantly in recent years. Although the fillers appear to be similar, their physical characteristics are not the same. New production technologies influence their physicochemical behavior in the skin and allow a rough differentiation among them. Understanding the physical-chemical properties of DF provides significant information about the expected clinical outcome. Therefore, it is important to find the control factors that may influence the effectiveness of DF at the site of application. In the literature there is a lack of studies about the correlation between the persistence in vivo of DF and their physical properties.

In this context the aim of this study was to find a correlation between the clinical performance of DF and their physical and chemical properties such as rheological properties.

Viscoelastic properties in terms of elastic and viscous moduli of different DF were determined by means of a small amplitude oscillatory shear test, and the dependence of viscosity upon shear rate was evaluated by a steady state shear test. Results were correlated with the clinical persistence of DF and the physical and chemical factors controlling the clinical performance were discussed.

MATERIALS AND METHODS

Materials

The HA-based dermal fillers, analyzed in this study, were obtained from commercial sources. Restylane Perlane is a product from Q-Med (Upsalla, Sweden); Belotero Basic, Belotero Soft and Belotero Intense are products from Merz Pharmaceuticals (Germany, Raleigh, NC, US); Juvederm Ultra 3, Juvederm Ultra 4 and Juvederm Voluma are products from Allergan (Irvine, CA, US); Teosyal Deep Line and Teosyal Ultra Deep are products from Laboratories Teoxane (Geneva, Switzerland).

Rheological measurements

The viscoelastic properties of materials have been evaluated on a rotational rheometer (Gemini, Bohlin Instruments, UK) using parallel plate geometry (PP15 Serrated cell).

Tests were carried out at the controlled temperature 37°C, by using a thermostatic bath. To avoid water evaporation, the humidity of the chamber containing the samples was controlled by a humidity Control Accessory.

Small-amplitude oscillatory shear experiments were performed to measure the unsteady response of the samples and hence the determination of their linear viscoelastic properties. In dynamic experiments the material was subjected to a sinusoidal shear strain:

$$\Upsilon = \Upsilon_0 \sin(\omega t)$$

where Υ_0 is the shear strain amplitude, ω is the oscillation frequency (which can also be expressed as $2\pi f$ where f is the frequency in Hz) and t time. The mechanical response, expressed as shear stress τ of viscoelastic materials, is intermediate between an ideal pure elastic solid (obeying Hooke's law) and an ideal pure viscous fluid (obeying Newton's law) and is therefore out of phase regarding the imposed deformation as expressed by:

$$\tau = G'(\omega)\, \gamma_0 \sin(\omega t) + G''(\omega)\, \gamma_0 \cos(\omega t)$$

where $G'(\omega)$ is the shear storage modulus and $G''(\omega)$ is the shear loss modulus.

G′ gives information about the elasticity or the energy stored in the material during deformation, whereas G″ describes the viscous character or the energy dissipated as heat.

The combined viscous and elastic behavior is given by the absolute value of complex shear modulus G^*:

$$\tan\delta = \frac{G'}{G''}$$

The ratio between the viscous modulus and the elastic modulus is expressed by the loss tangent:

$$\tan\delta = \frac{G'}{G''}$$

where δ is the phase angle (24).

The loss tangent is a measure of the ratio of energy lost to energy stored in the cyclic deformation. The phase angle, δ, is equal to 90° for a purely viscous material, 0° for a pure elastic material, and 0°<δ<90° for viscoelastic materials (25).

Preliminary amplitude sweep tests at a fixed oscillation frequency (1Hz) were performed on the materials to determine the strain amplitude at which linear viscoelastity is valid.

The samples were then subjected to a frequency sweep test in order to assess G′ and G″ based on frequency. The frequency range investigated was 0.01Hz-10 Hz.

Steady state shear test was also performed to evaluate the dependence of viscosity upon the shear rate.

RESULTS

The cosmetic outcome after a DF application may in vivo be influenced by their physical and chemical properties. Table I shows these properties for the DF studied. All the analyzed DF are based on HA, obtained by bacterial fermentation and cross-linked with 1,4-butane-diol diglycidyl ether (BDDE). The HA concentration varies from 20 mg/ml to 25.5 mg/ml. In particular, Restylane Perlane and Belotero Soft have the lowest concentration (20 mg/ml); while Belotero Intense and Teosyal Ultra Deep are characterized by the highest concentration (25.5 and 25 mg/ml).

Restylane Perlane is the only particulate DF with a number of particles of 10,000/ml. The other dermal fillers are monophasic HA gels. As reported in the literature, Belotero dermal fillers differentiate themselves from other DF in the manufacturing process, characterized by a dynamic, double cross-linking process yielding cohesive monophasic HA gel (26).

In Table II the DF rheological properties, in terms of the elastic and viscous modulus, tanδ, the complex rigidity modulus and viscosity of all samples at a frequency of 0.1 Hz are listed.

The results of the dynamic shear tests are reported in the following Figures 2-5, in particular in Figure 2 the elastic and the viscous modulus curves as a function of the oscillation frequency (mechanical spectra) at 37°C for the Teosyal Ultra Deep (Fig. 2a), Belotero Basic (Fig. 2b) and Belotero Soft (Fig. 2c), are reported, respectively and the values of G′ are fitted with a power law. From the mechanical spectra in Figure 2a it can be noted that G′ is quite frequency independent ($G' \propto f^{0,04}$) and G′ (274 Pa at 0.1 Hz) is higher than G″ (27 Pa at 0.1 Hz) by approximately one order of magnitude. Consequently, the value of tan δ, calculated as $\frac{G''}{G'}$, for Teosyal ultra deep is approximately 0.1. This sample behaves as *strong gel*.

From Figure 2b it can be noted that in this case also the elastic modulus is higher than viscous modulus and G′ and G″ are slightly frequency dependent ($G' \propto f^{0,29}$ and $G'' \propto f^{0,33}$). The loss factor is lower than 1 but higher than 0.1. This sample shows a *weak gel* rheological behavior. For Belotero Soft (Fig. 2c) G′ and G″ are strongly frequency dependent ($G' \propto f^{0,60}$ and $G'' \propto f^{0,56}$). The elas-

TABLE I - THE PHYSICAL PROPERTIES OF DERMAL FILLERS

Dermal filler	Company	Concentration mg/ml	Crosslinker	Number particles/ml	Needle size	HA SOURCE	Indication
Restylane Perlane	Q-Med	20	BDDE	10.000	27	BACTERIA	Medium to deep dermis and lips
Belotero Intense	Merz Pharmaceuticals	25.5	BDDE	ND	27	BACTERIA	Medium to deep wrinkles
Belotero Basic	Merz Pharmaceuticals	22.5	BDDE	ND	27	BACTERIA	Moderate to severe wrinkles and lips
Belotero soft	Merz Pharmaceuticals	20	BDDE	ND	30	BACTERIA	Superficial lines
Juvederm Ultra 3	Allergan	24	BDDE	ND	27	BACTERIA	Medium wrinkles and Lips
Juvederm Ultra 4	Allergan	24	BDDE	ND	27	BACTERIA	Medium to deep wrinkles, and lips
Juvederm Voluma	Allergan	24	BDDE	ND	23	BACTERIA	Deep dermis, add volume
Teosyal deep lines	Teoxane SA	25	BDDE	ND	27	BACTERIA	Deep dermis
Teosyal ultra deep	Teoxane SA	25	BDDE	ND	25	BACTERIA	Deep dermis, add volume

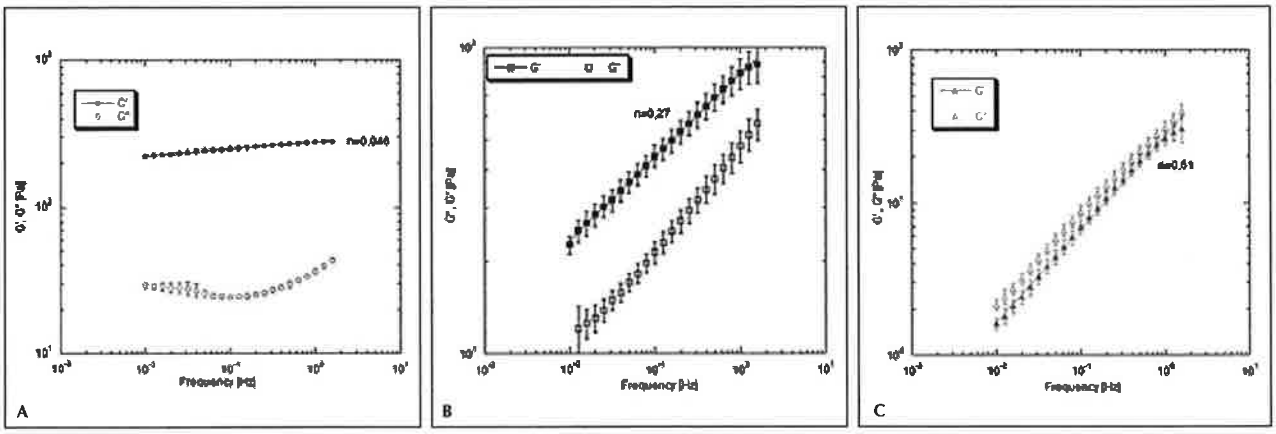

Fig. 2 - *Mechanical spectra of different dermal fillers at 37°C:* ***a)*** *Teosyal ultra deep,* ***b)*** *Belotero Basic,* ***c)*** *Belotero soft.*

TABLE II - VISCOELASTIC PROPERTIES OF HA-BASED DERMAL FILLERS AT FREQUENCY OF 0.1 HZ (T=37°C)

Sample	**G′ [Pa]**	**G″ [Pa]**	**Tanδ**	**IG*I [Pa]**	**η [Pas]**
Restylane	301.08 ± 8.55	47.07 ± 1.88	0.16 ± 0.03	304.74	230.35 ± 61.25
Belotero Intense	76.41 ± 7.90	26.50 ± 3.82	0.35 ± 0.12	80.88	1008.70 ± 115.06
Belotero Basic	44.21 ± 3.69	21.45 ± 1.85	0.49 ± 0.09	49.14	532.02 ± 229.04
Belotero Soft	6.93 ± 0.73	8.79 ± 0.76	1.27 ± 0.18	11,19	149.09 ± 46.19
Juvederm Ultra 3	173.28 ± 20.63	37.19 ± 4.29	0.21 ± 0.09	177.23	1629.90 ± 233.33
Juvederm Ultra 4	102.21 ± 11.46	29.81 ± 3.59	0.29 ± 0.04	106.47	1479.10 ± 75.41
Juvederm Voluma	603.14 ± 58.34	91.70 ± 10.79	0.15 ± 0.04	584.87	1033.40 ± 50.37
Teosyal deep line	177.63 ± 17.04	19.90 ± 1.89	0.11 ± 0.03	178.74	654.63 ± 169.03
Teosyal Ultra Deep	274.10 ± 6.39	27.28 ±0.92	0.1 ± 0.02	275.45	1242.5 ± 178.69

tic modulus (7 Pa at 0.1 Hz) is lower than the viscous modulus (8,8 Pa at 0.1 Hz) for a frequency lower than 1 Hz, but their values are very similar; the loss factor value indeed is greater than the unity. This sample behaves as an *entanglement network*.

A strong gel behavior was found also for Restylane Perlane, Juvederm Voluma and Teosyal Deep Lines. The elastic modulus comparison for these 4 samples, that behave as strong gels, is shown in Figure 3a. In detail, Juvederm Voluma has the highest magnitude of G′ (603 Pa at 0.1 Hz) and Teosyal Deep Lines has the lowest magnitude (178 Pa at 0.1 Hz). In Figure 3b, the elastic modulus comparison among the DF that behave as weak gels is reported. Belotero Intense, Belotero Basic, Juvederm Ultra 3, Juvederm Ultra 4 behave as weak gels. In this case Juvederm Ultra 3 and Juvederm Ultra 4 has the highest magnitude of G′ respectively 173 Pa and 102 Pa, while the lowest magnitude (44 Pa) is that of Belotero Basic.

In Figure 4, complex modulus, |G*|, at frequency 0,1 Hz for all samples is reported. The magnitude of complex modulus varies from 11.19 Pas to 584.87 Pas. The first value is related to Belotero Soft, the latter to Juvederm Voluma. It can be noted that the highest and lowest values of complex modulus, at low frequency, differ by almost two orders of magnitude.

A comparison between all the different DF loss factors as a function of frequency is reported in Figure 5. It can be noted that at 0.1 Hz Belotero Soft has the highest magnitude (1.27), and Teosyal Ultra Deep has the lowest magnitude (0.1).

The results of the steady state shear measurement in terms of flow curve, i.e. viscosity as a function of shear rate are reported in Figure 6. For all DF, viscosity decreases with increasing shear rate; in particular a power law relationship, $\eta = \kappa\dot{\gamma}^{-n}$, exists between viscosity and shear rate and the fitting is also shown in Figure 6. The value of

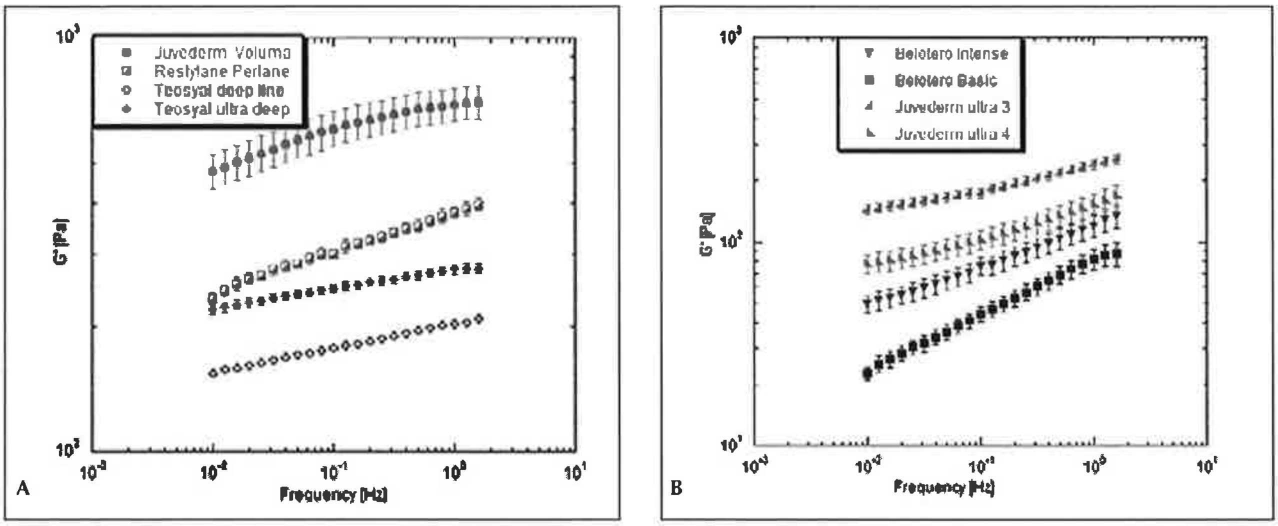

Fig. 3 - *Comparison of elastic modulus between different dermal fillers: 3* ***a)*** *strong gels, 3* ***b)*** *weak gels.*

k is indicative of the value of the viscosity when the shear is zero. Juvederm Ultra 3 has the highest value of k (336 Pa); instead, Belotero Soft has the lowest value of k (36 Pa). Moreover, all the curves, except for Restylane Perlane, present a value of n of about 0.7. For Restylane Perlane the viscosity profile shows a first region (with n = 0.91) at low shear rate (from 10^{-2} s^{-1} up to 10^{-1} s^{-1}). With increasing shear rate (> 10^{-1} s^{-1}), n decreases (n = 0.59).

Several studies on HA-based DF referred to an important rheological property, percentage elasticity, i.e. the proportion of elasticity in a cross-linked HA formulation. In this study the percentage elasticity, calculated as (100*G')/(G'+G''), as a function of frequency for all dermal fillers, is shown in Figure 7 and their values at frequency 0,1 Hz are listed in Table III. From Figure 7, it can be noted that the percentage elasticity of the DF ranges from 70% to 90% and only Belotero Soft presents a percentage elasticity of 40%.

It has been reported that the persistence of DF is inversely related to the percentage elasticity and the results are not intuitive but they suggest that the high elasticity alone cannot account for the persistence of DF.

In attempting to ascertain information about the persistence of dermal fillers, we considered the Wrinkle Severity Rating Scale (WSRS), that is a 5-point scale that can be used to assess the improvement of the onset of wrinkles following the injection of DF; in particular, we took into account the duration of dermal fillers at six months post-treatment. Table IV shows the 6-months improvement score WSRS and the success rate at 6 months post treatment for Restylane Perlane, Juvederm Ultra 3, Juvederm Ultra 4, Belotero Basic and Belotero Intense as found in the literature.

TABLE III - PERCENT ELASTICITY AND THE PRODUCT BETWEEN CONCENTRATION AND PERCENT ELASTICITY AT FREQUENCY 0.1 HZ, FOR ALL DERMAL FILLERS

Dermal fillers	% Elasticity @ 0.1 Hz	Concentration×% Elasticity @ 0.1 Hz
Restylane Perlane	86.48	1729.60
Belotero Intense	74.25	1893.38
Belotero Basic	67.33	1514.93
Belotero Soft	44	881.20
Juvederm Ultra 3	82.33	1975.92
Juvederm Ultra 4	74.42	1858.08
Juvederm Voluma	87.5	2099.28
Teosyal Deep Lines	89.9	2248
Teosyal Ultra Deep	90.9	2273.75

TABLE IV - SIX MONTHS WSRS IMPROVEMENT SCORE AND SUCCESS RATE FOR DIFFERENT DERMAL FILLERS

Dermal fillers	6 Months Improvement Score WSRS	6 Months Success rate (%)
Restylane Perlane	0.93	70
Juvederm Ultra 3	1.3	n.a.
Juvederm Ultra 4	1.4	88
Belotero Basic	1	81
Belotero Intense	1.78-1.9	n.a.

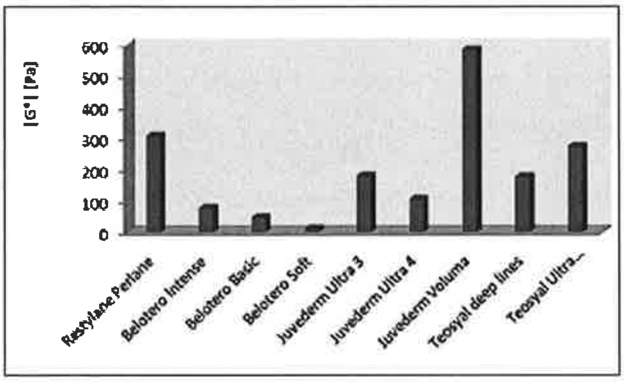

Fig. 4 - *A plot of the magnitude of complex modulus at frequency 0.1 Hz for different dermal fillers.*

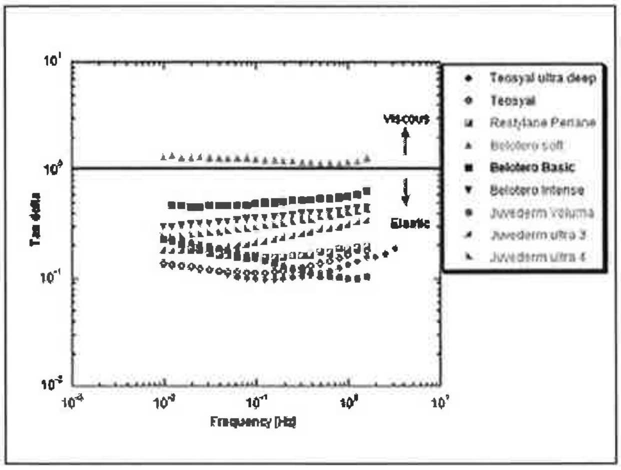

Fig. 5 - *Dependence of loss factor upon frequency for different samples.*

We want to underline that in the clinical studies which have been performed for the Restylane and the Juvederm products, touch-ups 14 days post initial treatment during the observation period were allowed, while for Belotero Basic only single treatment was allowed (27, 28, 29).

The improvement on the WSRS score seems to correlate with the polymer concentration for the aforementioned dermal fillers as shown in Figure 8. Indeed, there is a linear correlation between HA concentration and the improvement on the WSRS: polymer concentration increases as WSRS increases, meaning that the concentration significantly influences the persistence of dermal fillers but the concentration cannot be the only factor controlling the persistence of the DF since it does not take into account the importance of the cross-linking between the molecules. To consider the effect of both the percentage elasticity and the concentration as suggested in the literature (23) we considered another crucial parameter, the product between the concentration and percentage elasticity. The values of this product at frequency 0.1 Hz are reported in Table III.

In Figure 9 we have correlated the WSRS improvement score for the product between the concentration and percentage elasticity. The Figure shows that WSRS might be a function of the product of concentration and percentage elasticity. It has been suggested that the higher the product between the percentage elasticity and concentration, the higher the persistence of the DF (23).

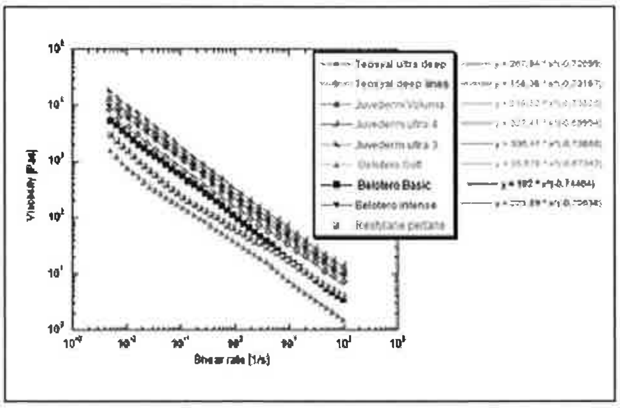

Fig. 6 - *Viscosity versus shear rate for all dermal fillers. The data are fitted with a power low curve (T=37°C).*

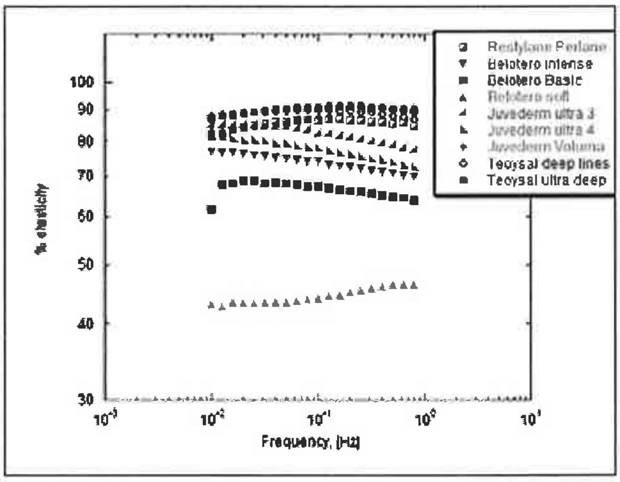

Fig. 7 - *Percentage elasticity as a function of frequency for different dermal fillers.*

DISCUSSION

The physical properties of HA-based DF may influence their clinical performance in vivo. The DF considered in this study are all HA-based. HA is an excellent biocompatible material, but it has a high affinity for water molecules and is quickly degraded by hyaluronidase. Because of this, following an injection of HA in normal skin,

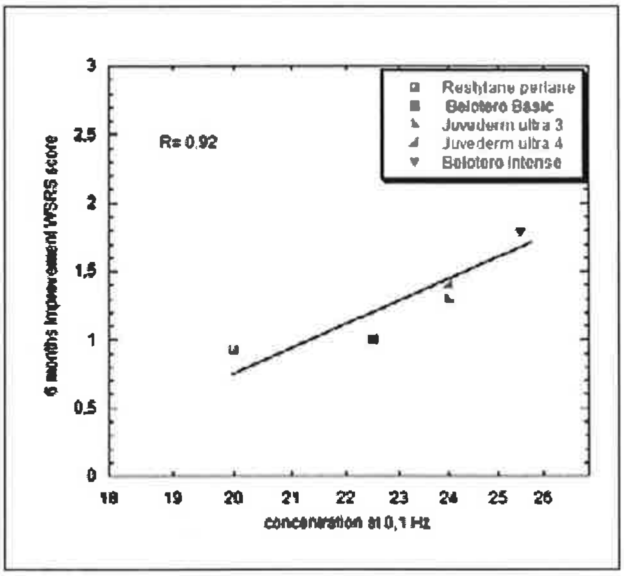

Fig. 8 - *Relationship between WSRS 6-months improvement score and concentration at frequency 0.1 Hz for Restylane perlane, Belotero Basic, Juvederm ultra 3, Juvederm ultra 4 and Belotero intense.*

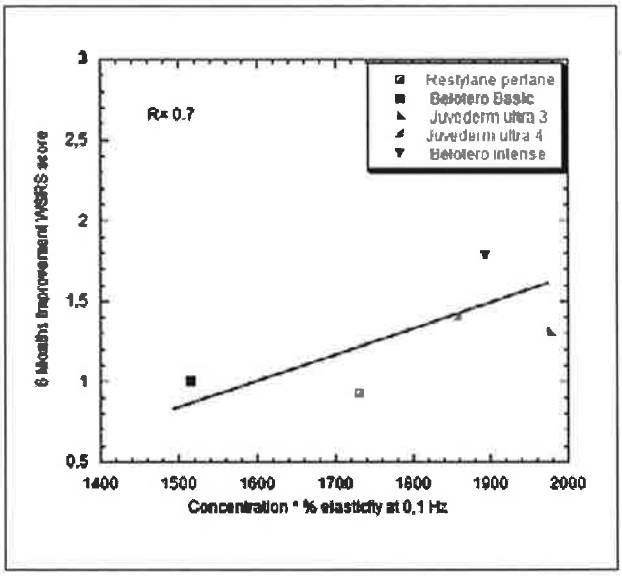

Fig. 9 - *Relationship between WSRS 6-months improvements score and the product of polymer concentration and percentage elasticity at frequency 0.1 Hz for Restylane perlane, Belotero basic, Juvederm ultra 3, Juvederm ultra 4 and Belotero intense.*

a fast degradation process often occurs. Therefore, HA has been chemically modified by cross-linking techniques to improve its biomechanical properties and to prolong the residence time in the implant site (27). The cross-linking among the polymer chains increases G′ at low frequency with a consequent increase in the aforementioned properties. The DF used in this study are all cross-linked with BDDE but differ in the production technology and not all are monophasic. The degradation is primarily cell-dependant because the degrading enzymes (mainly hyluronidase) are linked to cells; as dermal fillers are injected in the dermis the breakdown of them is supposed to be driven by macrophages engulfing the material. The surface- to- volume ratio is playing a large role in this process. Therefore particulate fillers due to their large surface area seem to be degraded faster than the monophasic or cohesive ones (31); this is supported by the 6-month clinical data (27, 28, 29). The rheological properties of cross-linked HA-based products vary greatly. The oscillation test has highlighted that the dermal filler exhibits a behavior of strong gels, weak gels and entanglement network. In particular, Restylane Perlane, Juvederm Voluma, Teosyal Deep Lines and Teosyal Ultra Deep behave as strong gels. In fact, they are characterized by an elastic response which is almost constant with the frequency; they have an elastic modulus one order of magnitude greater than the viscous magnitude and a loss factor of approximately 0.1. Because of the presence of permanent chemical cross-links among the molecules, under minor deformation conditions, these strong gels manifest the typical behavior of viscoelastic solids and the only way to accommodate stress is by elastic deformation. These gels have low tan δ values, high G′, percent elasticity and viscosity and consequently they possess the highest crosslinking density and so have the most rigid inner structure.

Moreover it has been reported that tan δ could be an indicator for the gel structure, the higher the value, the more flexible the HA filler and in some case a less rigid structure can prevent inflammatory reactions and immune response (32).On the other side Belotero Basic, Belotero Intense, Juvederm Ultra 3 and Juvederm Ultra 4 behave as weak gels and they have an elastic modulus lower than that of strong gels. Since the elastic modulus is proportional to the number of cross-linking points, this result indicates that the weak gels DF have a lower number of cross-linking points among the molecules and they probably possess a higher number of topologic interactions, entanglement among the chains, due to the higher molecule mobility. This behavior lies between that of solutions and that of strong gels. The mechanical spectrum of Belotero Soft is typical of entanglement networks. The viscous and elastic curves are strongly frequency-dependent and cross each other at 1 Hz. The elastic modulus is lower than that of the other DF, indicating that the cross-linking density is the lowest and the rheological properties are strongly influenced by the entanglements among the chains (33). Belotero is characterized by the highest flexibility, due to its novel manufacturing process. Under clinical considerations these gels are not characterized by a high stiffness and viscosity, making them able to be injected more superficially than the other fillers to obtain a comparable cosmetic result.These differences in rheological behavior are also highlighted by the different values of |G*| that describe the overall stiffness of the dermal fillers and that are related to the different cross-linking density.

Different viscoelastic properties are found within the same family of DF. This different rheological behaviour is desired because it is related to the different depth of site of injection and the different application of the product.

As regards the Belotero family, Belotero Soft, that is injected into the upper dermis and is an ideal substance for superficial wrinkles, is characterized by the lowest value of G′ and the highest value of tan δ; Belotero Intense, that is injected into the mid to deep dermis and is an ideal product for deeper lines and volume enhancement, is characterized by the highest value of G′ and of η and the lowest value of tan δ; Belotero basic, that is designed to be injected into the mid dermis and is indicated for moderate to deep wrinkles, is characterized by values of G′ and η and tan δ that are intermediate between those of Belotero soft and Belotero Intense.

As regards the Teosyal family, Teosyal deep lines, that is injected into the mid to deep dermal layer and is used to treat nasal furrows, is characterized by a G′ lower than that of Teosyal Ultra Deep, that is instead injected into the deep layer of the dermis and is designed for treating deep wrinkles.

The Juvederm family is also characterized by a wide range of elastic modulus, from 102 Pa to 603 Pa. Juvederm Ultra 3 is injected in the meid to deep dermis, medium to deep facial lines and skin depression may be treated; Juvederm Ultra 4 is used for volumizing and correction of deeper folds and wrinkles; instead, Juvederm Voluma is indicated for deep volume restoration to recontour the face and it is injected deeply into the deep dermis, subcutaneous or upper periosteal.

Furthermore, the rheological results demonstrate that as shear rate increases, viscosity decreases. For injectable materials, a shear thinning behavior is desirable since a low viscosity at high shear rate, as occurs when the materials go through the needle, enables easy injection of the gel. The high viscosity at low shear rate suggests a better ability to maintain the space.

Generally, the CLHA have high elastic to loss modulus throughout the entire frequency range, so to estimate the elastic contribution of the product, the percentage elasticity is generally (33-34) calculated. In the Teosyal family the percentage elasticity values are almost ugual; in the Juvederm family, the percentage elasticity values are in the range 77-88. As regards the Belotero family, the values are very different, in the range 44-75. All components of the Belotero family present the lowest values of percentage elasticity, making them able to be injected more superficially and which can be correlated to the lowest tissue reaction of the Belotero fillers as found by Taufig et al. (32).

One of the most important aspects of the clinical use of DF is their persistence in the human body. Generally, clinical trials are performed to evaluate the in vivo effectiveness.

Literature studies (35) show that the effect of treatment with Juvederm Voluma lasts up to 18 months; Teosyal Ultra Deep (36) and Restylane Perlane (37) lasts up to 9-12 months; Juvederm Ultra 3 and Juvederm Ultra 4 last up to 12 months (38). Belotero Basic and Belotero Intense last up at least 9 (27) to 18 months (26), like Belotero Soft that lasts up to 6 months (39).

In literature there are many studies oriented to the search for the ideal DF in terms of better persistence and clinical effectiveness but none of these studies correlate clinical performance to the physical properties. Here, we aimed to correlate the rheological properties to the clinical persistence. For this purpose, we considered an improvement score WSRS that is a measure of the relative effectiveness of DF at 6 months post-treatment, for Restylane Perlane, Belotero Basic, Belotero Intense, Juvederm Ultra 3 and Juvederm Ultra 4, to have an indication of the DF longevity, even if there are some differences in the clinical study design.

Our results demonstrate that polymer concentration might be a crucial parameter that influences the duration of dermal fillers; in particular, the increase in concentration is related to a higher WSRS and consequently determines better persistence. This behavior may be due to the fact that as the concentration of HA increases, the intermolecular associations increase.

Concentration alone cannot be the only factor controlling the performance of DF. Indeed, a major role in the duration of CLHA-based products is also played by the cross-linking among the molecules. For this reason, in agreement with the literature, for the analyzed DF, we found that another crucial parameter in the prediction of duration of dermal fillers is the product of HA concentration and percentage elasticity. Our results demonstrate that the increase in this product is related to the increase in WSRS, and consequently the persistence of dermal fillers in a linear manner. Nevertheless recent clinical data comparing Belotero Basic (27) and Restylane Perlane (28) e.g. show a longevity in cosmetic effect after 6 month in 81% and 70% of all patients. Both fillers are indicated to be implanted in the mid dermis but they show very different rheological properties, i.e. G′ and tan δ of Belotero Basic are 44,21 Pa and 0,49 respectively, those for Restylane Perlane are 301,08 and 0,16 respectively.

Rheological data can be a tool to have an indication on the efficacy and longevity of DF but it has to be considered that production technology, in-vivo-conditions, injector skills and experience influence them also significantly. From our rheological data we can conclude that the performance of DF is strongly correlated with the total concentration of CLHA in the material and with the product of concentration and percentage elasticity.

In conclusion, HA based DF have been characterized from a rheological point of view. In this paper we aimed to find a relationship between their physical and chemical properties and clinical performance in order to understand the characteristics of DF that may be responsible for clinical persistence in the dermis.

Qualitatively, when the CLHA materials were subjected to a sinusoidal stress, they exhibit a different rheological behavior as weak gels, strong gels and entanglement networks.

Moreover, also quantitatively our results highlighted that their rheologic properties are quite variable with respect to the magnitude of the viscoelastic moduli (G′, G″, G*), viscosity and percentage elasticity and rigidity of dermal HA fillers.

The data reported here indicate that a straightforward correlation exists between the persistence of CLHA products and polymer concentration, but concentration alone cannot be the only factor controlling the performance of DF, indeed the crosslinking among the molecules plays a major role in the duration of CLHA based products. Moreover, from our rheological results we can conclude that another important factor controlling the effectiveness of DF is the product between concentration and percentage elasticity.

Conflict of interest: None to declare.

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