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Abstract

Hyaluronic acid (HA) is an essential polysaccharide that is ubiquitous in the human body and has been in clinical use for over thirty years. Properties of HA make it an attractive substance for dermal fillers for managing volume loss in the aging face, such as its ability to bind large amounts of water, its natural presence in the skin and its low potential for adverse reactions. Three-dimensional facial recontouring through soft-tissue augmentation with fillers can provide patients with a fuller, youthful appearance that softens the appearance of aging. There are currently a number of dermal fillers available on the market based on injectable HA hydrogel. They differ in the specifics of their physical properties, and these differences have an effect on their final clinical performance. The physical properties of HA dermal fillers are determined by their HA concentration, cross-linking degree, gel hardness (elasticity), and injectability. A better knowledge of some of the physical properties of HA fillers can therefore be very useful for physicians in choosing the appropriate HA dermal filler for facial recontouring and/or a specific indication. In this study, the physical properties of seven selected HA dermal fillers were evaluated using the rheological testing method and compared.
Hyaluronic acid (HA) is a component of the natural extracellular matrix [1]. HA is found in high concentrations in many soft connective tissues, including the skin, the umbilical cord, the vitreous humour of the eye and the synovial fluid. This HA is composed of alternating units of D-glucuronic acid and N-acetyl-D-glucosamine. In the body, under physiological pH conditions, HA occurs in the form of salt, commonly referred to as hyaluronate.

It has been well established that HA can self-associate. Both intra- and intermolecular associations can exist under physiological conditions, creating a network-like matrix of HA chains. [2]. Thanks to its polyelectrolyte character, HA possesses an extraordinary visco-elastic property that is of crucial importance in its use in modern aesthetic medicine. HA is one of the most important stabilizing components of the extracellular matrix. Through various HA-binding proteins that are located on the surfaces of cells in the extracellular matrix, the substance helps to build the mechanically strong, three-dimensional network between the cells and collagen fibrils. It keeps cells in the matrix in their place [3], while the hydrophilic properties of HA ensure proper hydration of the tissues as well as intercellular and interfibrillar spaces.

Traditionally, HA was extracted from rooster combs and umbilical cords [4]. Since the early 1980s, HA is also commercially produced via microbial fermentation with Streptococcus zooepidemicus or Bacillus subtilis [5]. This method has been developed to meet the growing demand for HA in medical and cosmetic applications. HA has been successfully produced on an industrial scale with Streptococcus as the main producer.

Due to a unique combination of properties such as visco-elasticity, high hygroscopicity, magnificent biocompatibility, and non immunogenicity, HA is used in the treatment of osteoarthritis, cosmetics, ophthalmology, aesthetic medicine, surgery and wound healing, topical drug delivery, and tissue engineering [4,6,7]. The first comprehensive publication on the therapeutic use of HA was published in 1971 by Balazs [8].

Cross-linking is essential to the persistence of HA in the tissues [9]. Naturally occurring HA, free HA, floats free in solution, is not cross-linked and has a half-life of only 1 to 2 days. HA dermal filler manufacturers cross-link the macromolecules to form a hydrogel that is more resistant to degradation. These gels provide a more complex chemical structure and more robust physical barrier to the enzymatic and free radical degradation of dermal fillers once injected into the skin [9]. Balazs, Högberg and Laurent synthesized sulphated derivatives of HA, which they demonstrated to have anti-enzyme activities and to inhibit the proliferation of chicken heart fibroblast in vitro [10]. Cross-linked HA derivatives find application especially in aesthetic medicine, in treatment of osteoarthritis and in tissue engineering.

For several years, HA based dermal fillers have become the most successful response to the current massive demand for

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**Table 1: Dermal fillers tested in this study.**

<table>
<thead>
<tr>
<th>Product</th>
<th>Batch</th>
<th>Testing</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restylane Perlane®</td>
<td>11162-1</td>
<td>SAOS</td>
<td>Q-Med</td>
</tr>
<tr>
<td>Restylane Perlane®</td>
<td>10906-1</td>
<td>Extrusion force</td>
<td>Q-Med</td>
</tr>
<tr>
<td>Juvederm Ultra 2®</td>
<td>X24L722386</td>
<td>SAOS</td>
<td>Allergan</td>
</tr>
<tr>
<td>Juvederm Ultra 4®</td>
<td>S3OL759451</td>
<td>Extrusion force</td>
<td>Allergan</td>
</tr>
<tr>
<td>Teosyal Global Action®</td>
<td>TS30L-1140048</td>
<td>SAOS</td>
<td>Teoxane</td>
</tr>
<tr>
<td>Teosyal Ultra Deep®</td>
<td>TSUL-110201A</td>
<td>Extrusion force</td>
<td>Teoxane</td>
</tr>
<tr>
<td>Varioderm Plus®</td>
<td>P-4300</td>
<td>SAOS</td>
<td>Adoderm</td>
</tr>
<tr>
<td>Varioderm Plus®</td>
<td>P-1411</td>
<td>Extrusion force</td>
<td>Adoderm</td>
</tr>
<tr>
<td>Varioderm Subdermal®</td>
<td>S-4410</td>
<td>SAOS</td>
<td>Adoderm</td>
</tr>
<tr>
<td>Varioderm Subdermal®</td>
<td>S-2501</td>
<td>Extrusion force</td>
<td>Adoderm</td>
</tr>
</tbody>
</table>
non-surgical soft tissue augmentation. Intra-dermal injection of HA fillers are performed to fill wrinkles and to augment the volume of soft tissues such as lips, nasolabial folds and facial wrinkles [6]. The chemical cross-linked HA has been used as dermal filler for facial wrinkle treatment by injection, in order to give the skin more elasticity, improve tautness and enhance rehydration. For the use of HA, its elastic properties are of particular interest. These properties depend mainly on the molecular weight, the concentration of HA and the cross-linking of HA.

The chemical cross-linking of HA is generally performed involving the hydroxyl or the carboxyl groups of the HA chains. The two cross-linkings agents that are currently in use, 1,4-butanediol diglycidyl ether (BDDE, used in Restylane, Juvederm, Teosyal, and Varioderm Lips & Medium), and divinyl sulfone (DVS, used in Hylaform, Prevelle Lift, Varioderm Plus, and Varioderm Subdermal) react with the hydroxyl sites on the HA chains.

Relevant specifications for the efficacy of HA dermal fillers
When HA polymer chains are cross-linked, a three-dimensional network of the gel is formed. A large three-dimensional gel can not be used as a dermal filler, the gel is therefore fragmented into smaller gel particles, which allow the gel to be injected. Often, some manufacturers of dermal fillers add non-cross-linked HA as a lubricant to facilitate the injectability [11,12]. They differ in HA concentration, cross-linking degree, particle size, swelling capacity, amount of non-cross-linked HA present in the formulation and the elastic modules [11,12]. Due to different HA dermal fillers available on the market, it is sometimes not easy to find the best filler for a specific purpose. A better knowledge of some of the specifications of HA fillers can therefore help physicians in choosing the appropriate HA dermal filler for a specific indication.

The following specifications of HA dermal fillers strictly affect their final clinical performance [9,13]:
- Concentration of HA
- Degree of cross-linking
- Quantity of HA cross-linked vs. non-cross-linked
- Duration of filling effect
- G’ (elastic modulus)
- Injectability (extrusion force)

The elastic modulus G’ (pronounced G prime) of the product is one of the most important considerations [13]. G’ is a measurement of gel hardness.

Materials and Methods

Materials
For the HA dermal filler products that were tested for the evaluation of their physical properties, see table 1.

| Elasticity: | The ability of a material to return to its original shape after being deformed. |
| Elastic modulus G’: | The G’ value indicates the deformation energy contained in the matter during deformation. When the stress or pressure is removed, this energy is available to restore the matter to its original shape, fully or partly reversing the deformation. |
| Viscosity: | Viscosity is a measure of the resistance of a fluid which is being deformed by either shear or tensile stress. |
| Viscous modulus G’’: | The G’’ value indicates the energy used during deformation of a material, and is thus the share of the deformation energy lost during deformation. |

Table 2: Rheological terms used to describe physical properties of materials.
Rheology

Rheology is the branch of physics that deals with the deformation and flow of materials [14]. Rheology builds on the correlation of the deformation of a material and the applied stresses and deals in particular with flows. In polymer solutions, flow processes are often extremely complex. The viscosity of a polymer solution depends on the rate of deformation or strain and is characterized by the occurrence of elastic phenomena [15]. In Newtonian fluids, such as oil and water, the viscosity does not change with the flow rate or strain rate.

The physical properties of materials are described using a variety of rheological terms (see table 2).

Small amplitude oscillatory shear

Small amplitude oscillatory shear (SAOS) is a rheological method to determine the visco-elastic properties of materials [16]. An advantage of this method is that shear-sensitive structures like hydrogels can be characterized free of destruction, because over the whole measuring range the amplitude is kept very small, in order to work within the so called linear visco-elastic region.

In this test, a sample of the substance is placed between two plates and exposed to sinusoidal deformation γ at radial frequency ω (figure 1). There is a delay between the excitation function and the response, known as phase shift δ (figure 1).

With an ideal elastic solid, the phase angle is δ = 0°. Newtonian fluids have a phase angle of δ = 90°, while a visco-elastic substance is characterized with a phase angle δ of between 0° and 90°.

The elastic modulus G' and the viscous modulus G'' can be calculated from the results measured during the experiment [17]:

\[ G' = \frac{\sigma_0}{\gamma_0} \cdot \cos\delta \]
\[ G'' = \frac{\sigma_0}{\gamma_0} \cdot \sin\delta \]

The ratio between the viscous modulus and the elastic modulus corresponds to the loss tangent (loss factor) tan δ, and thus describes the ratio between the elastic and the viscous share of a polymer fluid.

\[ \tan \delta = \frac{G''}{G'} \]

If the loss tangent δ is greater than 1, the material is predominantly viscous, and if it is smaller than 1, the material is predominantly elastic. In order to distinguish between different visco-elastic fluids, it is therefore necessary to know the tan δ.

In order to determine the properties of shear-sensitive materials such as dermal fillers made from cross-linked hyaluronic acid, the SAOS method is particularly useful.

The SAOS experiments were performed with a HAAKE Rheo-Stress 1 rheometer (from Thermo Fisher Scientific, Process Instruments, Karlsruhe, Germany) at 25°C. All dermal fillers were examined with plate/plate measuring geometry (D = 35mm) using a gap of 1 mm and at a frequency range of 100 to 0.10 rad/s. The elasticity modulus G', the viscous modulus G" and the loss tangent tan δ were determined from the measurements at a frequency of 0.63 rad/s.

Extrusion force

The physician must apply to inject the HA filler through a thin needle and into soft tissue, therefore the extrusion force of HA dermal fillers is clinical relevant.

After the cross-linking of HA, it results in a large gel mass that must be broken up into gel particles, so that it may pass...
through a thin needle. The newer HA fillers that use advanced sizing techniques to achieve a broad distribution of gel particle sizes and a smooth consistency, have the advantage that less uncross-linked HA is needed to achieve injections with more even flow. Therefore, such products provide a higher percentage of cross-linked HA that can be implanted into the skin, which may improve persistence and clinical outcomes [9].

The extrusion force of prefilled, disposable syringes can be determined by means of a testing device according to DIN EN ISO 7886-1 (Annex G: Determination of forces required to operate the plunger).

The extrusion force of prefilled syringes was measured with a Zwick/Roell Z005, Fmax 200N testing device (from Zwick Roell AG, Ulm, Germany). The measurements were performed with a type 27G needle (TSK Laboratory, Japan).

Results

In In the past, scientists and physicians have discussed the effects that certain properties of dermal fillers might have on the actual filling effect of a product. It has long been thought that the key parameters for the clinical application of dermal fillers were the HA concentration and the size of the gel particles in the product [18]. The clinical evaluation of Restylane and Perlane (two products of identical chemical composition with a mean particle size of 300 µm and 650 µm respectively) showed however that the particle size does not affect the time of retention in the body [19, 20]. For cross-linked HA, its concentration is the decisive factor for high efficacy (with regard to filling effect and retention time). This does however not apply to non-cross-linked HA, as such substances are decomposed in the body within only a few days [9]. Table 3 shows the concentrations of the selected HA dermal fillers and their measured physical properties. The gel hardness (elastic modulus G') plays a major role for the efficacy of a HA filler. This parameter is determined by the HA concentration, the molecular weight of the HA and the share of non-cross-linked HA in the end product. The elastic modulus can be used to determine the suitability of a HA filler for a specific application. HA fillers with greater elastic modulus have a better filling effect and a longer retention time in soft tissue.

The measured elastic modulus values (table 3) indicate that Juvederm Ultra 2 is the lowest of the selected HA fillers (G' = 75.5 Pa), followed by Teosyal Global Action (G' = 140 Pa), Restylane Perlane (G' = 695 Pa), Varioderm Plus (G' = 940 Pa) and Varioderm Subdermal (G' = 2190 Pa). The elastic modulus of Varioderm Subdermal is thus three times greater than that of Restylane Perlane, while the elasticity modulus of Varioderm Plus is seven times greater than that of Teosyal Global Action, and even twelve times greater than that of Juvederm Ultra 2 (figure 2). Given the high elasticity of the Varioderm fillers, these products can achieve better volumizing effect and a longer lasting augmentation in the soft tissue [21, 22]. In addition, the high elasticity of these fillers means that less product is required to achieve the same effect as with less elastic dermal fillers.

Varioderm dermal fillers are produced by means of monophasic particle technology (MPT). The tests show that the Varioderm fillers offer better injectability than the other dermal fillers,
Extrusion force versus extrusion required to depress syringe plunger of the tested HA dermal fillers at a constant rate.

The measured extrusion forces (figure 3) show that Teosyal Ultra Deep requires by far the greatest extrusion force ($F_{\text{max}} = 57.06$ N), followed by Juvederm Ultra 2 ($F_{\text{max}} = 32.74$ N) and Restylane Perlane ($F_{\text{max}} = 19.35$ N). In contrast, Varioderm Subdermal ($F_{\text{max}} = 17.02$ N) and Varioderm Plus ($F_{\text{max}} = 10.00$ N) require a significantly smaller extrusion force. The maximum extrusion force of Teosyal Ultra Deep is about three times greater than that of Varioderm Subdermal and even six times greater than that of Varioderm Plus. In addition, curve progression of Teosyal Ultra Deep shows that the product does not possess a homogeneous mixture (figure 3).
Currently on the market available HA dermal fillers have their limitations related to clinical performance, persistence and ease of injection. Therefore, a better knowledge of the chemical and physical properties of HA dermal fillers may help physicians in choosing the appropriate HA dermal filler for facial enhancements [9].

The results of this rheological study indicate that seven selected HA dermal fillers possess a range of physical properties. Varioderm® products have significantly better elastic properties and are easier to inject than the other evaluated fillers (Teosyal, Juvéderm and Restylane). This is mainly due to the innovative monophasic particle technology (MPT) used in the production process for the Varioderm fillers. The optimized physical properties of the Varioderm fillers therefore allow for better clinical results. Understanding of physical properties of dermal fillers and their potential impact on clinical performance may better equip the physicians to select appropriate HA filler products based on patient needs and desired outcomes [23].

### References