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Original Article

Heat Influence on Different Hyaluronic Acid Fillers

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DISCLOSURE: **ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.**

Facial volume deficits, providing long-term facial aesthetic enhancement outcomes for the signs of aging and/or facial contouring. Numerous hyaluronic acid (HA) fillers seem to have similar characteristics, although their properties regarding rheology, viscoelasticity, heat resistance are different in many ways. The resistance heat degradation is important when hyaluronic acid fillers and energy-based devices are going to be used sequentially. Our objective was to determine the characteristics of HA gels in terms of heat resistance. Degradation of the gels was measured as a change of surface area of the sample. Five types of HA fillers, chosen from most common products on the market for temporary correction of congenital and acquired soft tissue deficits of the face via intradermal or subcutaneous injection: 20mg/ml HA-BDDE, 20mg/ml HA-BDDE, 20mg/ml HA-BDDE, 25 mg/ml HA-BDDE, 28mg/ml HA-PEG were tested in this study. Even though the three dermal fillers contained the same concentration of HA and were cross-linked with the same cross-linking agent, they were produced by different manufacturers using different technologies developed by individual companies. We tested *in vitro* resistance to heat degradation using Celltibator GT (Medikan Co., LTD, Seoul, Rep. of Korea) and Autoclave (Medotti 22L PRO, Poland). All of the HA fillers samples (0,3 ml) were placed on the petri dishes and put into the autoclave for 10 minutes (temp. 72,4°C). Three of the gels samples (20mg/ml HA-BDDE, 25mg/ml HA-BDDE, 28mg/ml HA-PEG) each 0,3ml were placed into Celltibator for 10 min, temp. 55,2°C degree, centrifugation: 30 RPM. Centrifugation was used to imitate the behaviour of the fillers under the conditions of forces acting on it in the tissue (stress under the influence of facial expressions, exercises, etc.). The temperatures used during this test correspond with commonly used heat-based devices, such as radio-frequency devices (about 45°C), infrared (about 55-65°C) and HiFU (about 70-75°C). Before and after each test pictures of the samples were taken. Heat degradation of the HA samples was measured by comparing (before and after) the changes of the surface area of samples on the petri dishes (on the graph paper). The 28-mg/ml HA-PEG gel filler demonstrated greater resistance to heat versus the 20- mg/ml and 25-mg/ml BDDE gel fillers. The 28-mg/ml HA-PEG, demonstrated in both test (celltibator with/without rotation and autoclave) greater resistance to heat in terms of deformation / thermal degradation and change of surface area. Selection of dermal filler with the right rheological properties is a key factor in achieving a natural-looking long-lasting desired aesthetic outcome. Hyaluronic acid fillers combined with energy-based devices are frequently used sequentially during the same session, however, in some cases it might cause thermal damage of HA. Caution is advised in using IR over recently injected filler (selection of dermal fillers is crucial in this case). Study limitations include use of *in vitro* model and lack of inflammatory response in an *ex-vivo* model.

INTRODUCTION

Facial rejuvenation treatments with hyaluronic acid (HA) fillers are safe and effective aesthetic procedures for patients seeking to maintain a youthful appearance. The number of non-surgical procedures using fillers based on hyaluronic acid has grown to almost 3 million worldwide and the growing trend continues (1). The appeal of these procedures is due to the immediate aesthetic effect and the relatively short recovery time involved (2, 3). The commercial success and popularity of soft tissue fillers based on hyaluronic acid is due to their biocompatibility and reversibility. The production technology, but also the type of cross-linking agent and the cross-linking density of hyaluronic acid can affect not only the durability of the treatment effect in vivo, but also the safety profile of dermal fillers. In particular, the crosslinking parameters play a major role in determining the physical and chemical properties including rheological and swelling ratio of the hydrogel

features that plays a major role for clinical applications (4, 5, 6). Several crosslinking technologies were introduced to the market last years: 1,4-Butanediol diglycidyl ether (BDDE), divinyl sulfone (DVS), hexamethylenediamine (HMDA) and polyethylene glycol diglycidyl ether (PEGDE) (6, 7, 8, 9). HA products' success stems from several characteristics: negligible allergic risk, and the injective procedure is quick to perform. Results of HA treatments are acceptably long-lasting (average duration of action is 6 months), and what is important from doctor an patients perspective - easy to correct, fully reversible in the event of adverse effects thanks to the products' biodegradability (10). Hyaluronic acid injections enhance tissue hydration and enrich the dermis for one of the main ECM constituents; they increase the biosynthetic capacity of fibroblasts and stimulate synthesis of new extracellular compounds (11). Currently available HA-based injectables differ not only in the source and concentration of HA but also for in the modification/ stabilization method and, more interesting still, in their rheology. Even with the same concentration of hyaluronic acid and the same cross-linking method, the products may have different physico-chemical properties. This may be due to the different molecular weight of HA, the amount of cross-linking agent or the cross-linking density.

Crosslinking agents are one of the most successful chemical modifications of HA, and act as chemical species connecting two sections of HA chains in a bridge-like way. The main crosslinking species currently employed are 1,4-butanediol diglycidyl ether (BDDE) and polyethylene glycol diglycidyl ether (PEGDE). With regard to fillers, the reaction between the crosslinking agent and HA is the formation of ether bonds, as they are stable in physiological conditions in the dermis (12). The vast majority of HA dermal fillers available on the market are cross-linked with BDDE (12). BDDE is furthermore less toxic than other ether-bond crosslinking agents such as divinyl sulfone and is biodegradable. Upon degradation, both unreacted BDDE and cross-linked HA break down into harmless or naturally occurring substances and byproducts. The favorable clinical safety profile of BDDE-HA is supported by almost 30 years' worth of clinical and biocompatibility data (13). PEGDE is a linear or branched polyether, with a wide range of applications in biomedicine thanks to its nontoxicity and non-immunogenicity (12). Similar to BDDE, hyaluronic acid crosslinking with PEG is based on the formation of ether bonds (14). PEGDE consists of a mixture of oligomers of different lengths; weights, non-crosslinked HA is polymer too and as a result of combination and cross-linking, we obtain a mixture of two polymers with unequal spacers between the HA chains, as opposed to simpler molecules such as BDDE. The effective crosslinker ratio for PEG appears lower than that of BDDE, which may be responsible for differences in rheological properties and swelling rate of the two compounds (12, 15). PEGDE cross-linking also affects the rheological behaviour of PEGDE-HA hydrogels display improved elasticity as compared to BDDE-HA at the same molar concentrations (16). When comparing swelling ratios, for hyaluronic acid cross-linked with either BDDE or PEGDE, lower swelling rates have been reported for PEGDE as compared with BDDE-based formulations both in vitro and in vivo (15, 16). Furthermore, PEGDE-HA has been seen to possess a greater resistance to degradation by hyaluronidase than that of BDDE-HA.

Taking into account the huge number of HA dermal fillers available on the market and the differences resulting from both HA concentration and various cross-linking methods, our objective was to determine the characteristics of HA gels in terms of heat resistance. Heat resistance may be an important indicator helping the doctor select the appropriate product for a given treatment or combination therapy using heat emitting devices in the same session with HA dermal fillers.

MATERIALS AND METHODS

For the purposes of the study, we selected five types of HA cross-linked fillers, chosen from most common products on the market for temporary correction of congenital and acquired soft tissue deficits of the face via

intradermal injection. Four dermal fillers crosslinked with BDDE: 20mg/ml HA-BDDE, 20mg/ml HA-BDDE, 20mg/ml HA-BDDE, 25 mg/ml HA-BDDE, and one product crosslinked with different technology: 28mg/ml HA-PEG were tested in this study. Three of the selected products contained the same concentration of HA (20mg/ml) and were cross-linked with the same cross-linking agent (BDDE), but they were produced by different manufacturers using different technologies developed by individual companies. In the further part of the work they will be called BDDE I, II, III. We do not specifically provide detailed product characteristics or manufacturer's names, the purpose of the study is only to check the response of fillers based on hyaluronic acid to heat, and not to evaluate individual products.

Celltibator GT (Medikan Co., LTD, Seoul, Rep. of Korea) and Autoclave (Medotti 22L PRO, Poland) were used to test the resistance to heat degradation of the tested HA fillers.

The temperature range in which the measurements were made was in the range of 55.4 - 72.4°C. Such selection was not accidental but correspond with commonly used heat-based devices, such as radio-frequency devices (about 45°C), infrared (about 55-65°C) and HiFU (about 70-75°C). In addition, we used centrifugation to imitate the behaviour of the fillers under the conditions of forces acting on it in the tissue (stress under the influence of facial expressions, exercises, etc.).

Degradation of the gels was measured as a change of surface area of the sample (graph paper & graphing program for area calculation).

Study design

This study was design to shown that the choice of dermal filler, especially in the context of protocols combining different technologies, especially a heat emitting device, can be a key factor in achieving a natural-looking, safe, long-lasting desired aesthetic result.

This work was intended to examine the behaviour of hyaluronic acid-based hydrogels in the presence of heat in a very simple way, and is therefore less accurate than when more advanced analytical methods were used. Changes of surface area is not a perfect or optimal observation tool, but sufficient to observe changes, verify hypotheses and guide further research. These results should be treated as an introduction - proof of concept. It is reasonable to conduct more advanced research showing the influence of temperature on the physicochemical properties of gels, e.g. on rheology.

Celltibator without centrifuging

Three HA fillers (20-mg/ml HA-BDDE; 25-mg/ml HA-BDDE; 28-mg/ml HA-PEG) samples (0,3 ml) (Fig. 1) were placed on the petri dishes and put into the Celltibator for 10 minutes (temp. 55,2°C) without centrifuging. Pictures before and after were taken.

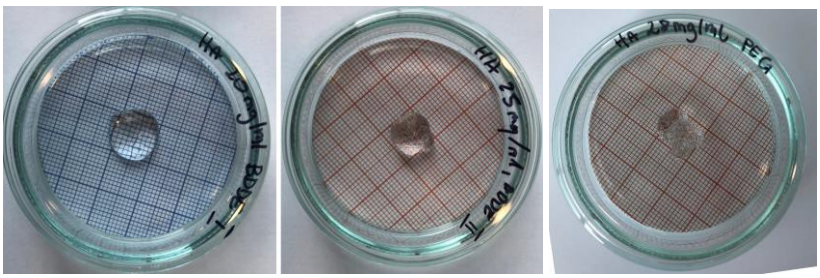

Fig. 1. *HA samples of 20-mg/ml HA-BDDE; 25-mg/ml HA-BDDE and 28-mg/ml HA-PEG; each 0,3 ml, before heating test in the Celltibator.*

Celltibator with centrifuging

Two HA fillers (20-mg/ml HA-BDDE; 28-mg/ml HA-PEG) samples (0,3 ml) (Fig. 2) were placed on the petri dishes and put into the Celltibator for 10 minutes (temp. 55,2°C) with centrifuging (30 rpm). Pictures before and after were taken.

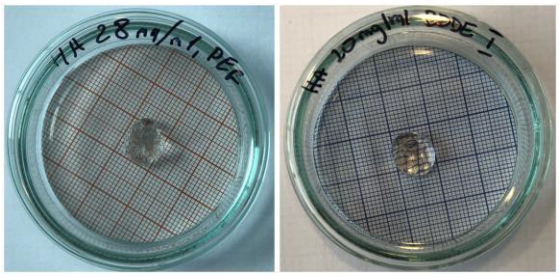

Fig. 2. *HA samples of 20-mg/ml HA-BDDE and 28-mg/ml HA-PEG; each 0,3 ml, before heating and centrifuging in the Celltibator.*

Autoclave

All samples of HA fillers indicted for temporary correction of congenital and acquired soft tissue deficits of the face via intradermal or subcutaneous injection, three of them contained the same concentration of HA and were cross-linked with the same cross-linking agent, but they were produced by different manufacturers using different technologies developed by individual companies (20mg/ml HA-BDDE, 20mg/ml HA-BDDE, 20mg/ml HA-BDDE, 25 mg/ml HA-BDDE, 28mg/ml HA-PEG) were placed on the petri dishes (0,3 ml of each) and put into the Autoclave (Medotti 22L PRO, Poland) for 10 minutes (temp. 72,4°C) (Fig. 3). Pictures before and after were taken.

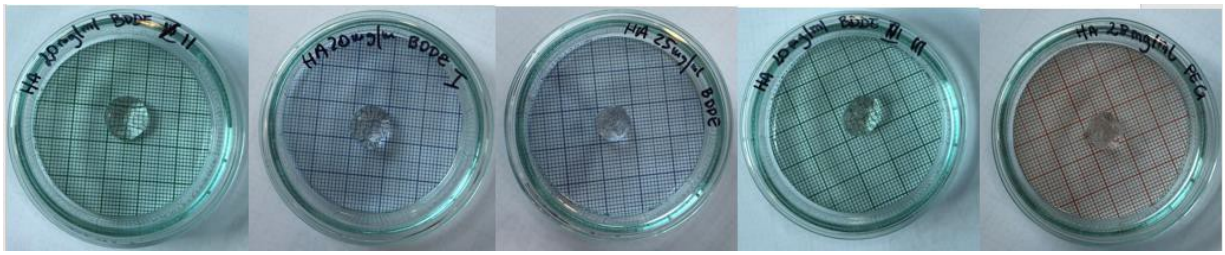

Fig. 3. *Samples of HA fillers (0,3 ml each), before heating in the autoclave*

RESULTS

As shown in Tables I and II during the test proceeded in the celltibator (temp. 55,2°C) with and without centrifuging, change of surface area of the samples was observed. In case of the combined test – heating and centrifuging for the sample of 20 mg/ml HA-BDDE gel, the degradation was significant.

Table I. *Results of heating of HA samples (20-mg/ml HA-BDDE; 25-mg/ml HA-BDDE; 28 mg/ml HA-PEG).*

	Before (cm^2)	After (cm^2)	Change (%)
20 mg/ml BDDE I	1,663	1,715	3,1
25 mg/ml BDDE II	1,142	1,188	4,0
28 mg/ml PEG	1,502	1,502	0,0

Table II. *Results of heating and centrifuging of HA samples (20-mg/ml HA-BDDE and 28- mg/ml HA-PEG).*

	Before (cm^2)	After (cm^2)	Change (%)
20 mg/ml BDDE I	1,155	1,500	29,9
28 mg/ml PEG	1,309	1,366	4,3

All tested samples of HA fillers after 10 minutes in autoclave in the temperature of 72,4°C demonstrated changes of surface area (Table III). However, it is worth to underline that the result linked with the PEG cross linked gel was the lowest. The surface change of the BDDE cross linked gels were in range of 4,1% - 18,0% in comparison to PEG-HA 3,6%.

Table III. *Results of heating of HA samples (autoclave; 10 min; 72,4°C). BDDE I, BDDE II, BDDE III – three dermal fillers with the same concentration of HA, produced by different manufacturers using different technologies developed by individual companies.*

	Before (cm^2)	After (cm^2)	Change (%)
20 mg/ml BDDE I	1,364	1,610	18,0
20 mg/ml BDDE II	1,224	1,275	4,1
20 mg/ml BDDE III	1,265	1,345	6,3
25 mg/ml BDDE	1,000	1,600	6,0
28 mg/ml PEG	1,321	1,368	3,6

DISCUSSION

This study has shown that the choice of dermal filler, especially in the context of protocols combining different technologies, including a heat emitting device, can be a key factor in achieving a natural-looking, safe, long-lasting desired aesthetic result. Hyaluronic acid-based fillers in combination with energy-powered devices are increasingly used in the same session, but as always, the question arises whether such combinations do not cause thermal degradation of HA. This also applies to patients who want to take advantage of treatments using energy-using devices, and have previously undergone HA filler treatments. Therefore, it is very important for us to choose a product that is as resistant to temperature as possible. Tissue temperature during EBD (Energy Based Device) treatments ranges from 45 to 70°C, and the human face is not static. Knowing this, we decided to check *in vitro* how the most popular fillers on the market will behave at a temperature of 55 to 70°C, also during the stress condition of forces acting on them. Of course, we are aware of the limitations of this test, our objective was to determine the characteristics of HA gels in terms of heat resistance, even using very basic methods.

In this study in a very basic way, the heat resistance of five HA hydrogels has been evaluated. The five tested products differ for hyaluronic acid content and cross-linker type and concentration. The aim of this study was to assess, *in vitro*, how the features of a filler can influence the product heat resistance, and in consequence the duration over time of the implant used together with EBDs. What is an interesting observation, and what should be the basis for further, more advanced research, is the differences of results between the two types of cross-linking agents. The results obtained showed that all the tested products were sensitive to heat degradation, but the degradation percentage obtained in the experimental conditions was much lower for HA filler crosslinked with polyethylene glycol diglycidyl ether (PEGDE). The results could lead to the conclusion that the degradation level was cross-linker dependent. In particular, the use of PEG as a crosslinking agent is an innovation in the biomedical field and makes interesting the study of PEGylated fillers in order to

investigate their behaviour in the presence of heat in terms of improved stability and preserved biocompatibility when used together with EBDs. A filler used together with EBD should be endowed with long term stability but, at the same time, with plasticity and should be biocompatible.

In conclusion, we found a correlation between cross-linker type and it heat degradation resistance, but due to study limitations further investigations are needed.

CONCLUSIONS & FUTURE IMPLICATIONS

The study results could provide evidence on the clinical performance of HA fillers used together with heat emitting devices for facial rejuvenation. Summarizing the results point to the conclusion that the heat resistance of HA fillers is cross linking agent depended, and selection of dermal fillers is crucial in this case of using IR, RF, HiFU (or any other heat emitting device) over the injected filler. To minimize the possible degradation of HA fillers, combination treatments with HA fillers and EBDs can be also sequentially performed using EBD before and no after dermal filler. This is a good method, but it may not always be effective, especially in cases of multiple treatments performed at short intervals on the same patient. Therefore, it seems important to choose the right product ensuring a natural, optimal aesthetic effect combined with the highest possible safety profile.

The creation of cross-linked forms of hyaluronic acid made it possible to bypass two basic problems related to the use of this substance as a filler: rheological properties (non-cross-linked hyaluronic acid does not have sufficient cohesion and viscoelasticity) and resistance to enzymatic degradation. Non-cross-linked hyaluronic acid half-life in tissues ranges from less than 1 to several days (17), which is due to the presence of hydrolases such as hyaluronidase, which cause very rapid degradation of this glycosaminoglycan. It seems that cross-linking by looping hyaluronic acid molecules limits its availability for naturally occurring hyaluronidase, and factors causing mechanical degradation of the filler contribute to damage to its structure, which increases the possibility of enzymatic degradation. The authors' clinical observations resulting from several years of clinical practice indicate a faster disappearance of fillers based on hyaluronic acid in areas with intense facial expressions (lips, areas of nasolabial folds, etc.) compared to areas with negligible mechanical stress (infraorbital areas, temporal spaces, etc.). Our own observations indicate that the same filler used in anatomical areas subjected to various mechanical stress provides a satisfactory aesthetic effect in a very wide period of time (about three times longer in the case of areas with limited mimicry). This is a preliminary observation that requires in-depth, large-scale research using tissue imaging methods. Thermal degradation using suddenly occurring high temperatures (using previously described popular energy based devices) can lead to the same result as intense mechanical stress of the product, leading to an increase in its sensitivity to endogenous hyaluronidase. The occurrence of thermal trauma to the tissue itself, causing subsequent inflammation in the immediate vicinity of the filler, which results in increased cellular activity expressed, among others, by increased release of endogenous hyaluronidase (18) seems to be an additional important factor beside the mechanical stress.

Although this study was conducted in a very basic way, the results are promising, however an in-depth knowledge of the behaviour of hyaluronic acid-based fillers both *in vitro* and in vivo is required. Increased heat resistance of PEGylated HA dermal fillers should be subjected to further testing.

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Conflicts of Interest: Paweł Kubik is a scientific external consultant of Matex Lab.
The authors declare no other conflicts of interest.

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