Hyaluronic acid-based *fillers*: how to choose *the right one?*

BIOFORMULA
ADVANCED BIOLOGICAL RESEARCH
The Italian Beauty Solution

Scientific Disclosure Article reserved for Physicians. Edited by the Scientific and Technical Department of Bioformula®

Hydrogel based on hyaluronic acid (HA) are extensively used in many biomedical applications, including regenerative medicine, aesthetic medicine and drug delivery.

HA is a natural constituent of the extracellular matrix; in human connective tissue, it has an important structural role, due to its high hydrophilicity and molecular weight; it is hydrolysed and degraded by hyaluronidases [1,2].

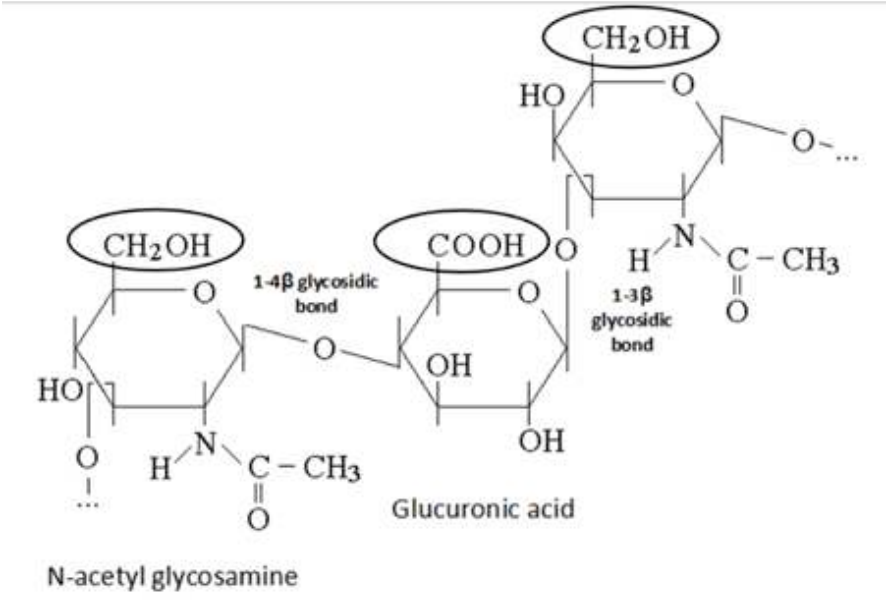
Thanks to its biocompatibility, chemical-physical and biological properties, and easiness of chemical functionalization, HA has immediately acquired an increasing interest among researchers for its potential used in soft tissue replacement and augmentation, surgical procedures and diagnostics.

For a clinical application of HA, degradation should be controlled through cross-linking or conjugation to stabilize HA and preserve its fundamental features [3].

Cross-linking with DVS or BDDE?

Two possible methods to modify HA are derivatization and cross-linking; both funtionalizations can be achieved through reactions between the available functional groups of HA (-COOH, -OH).

Chemical Structure of Hyaluronic Acid



When divinyl sulfone is used as a cross-linking agent, the hydroxyl (-OH) groups on the HA chains reacts under alkaline conditions to yield stable hydrogels containing sulfonyl-bis-ethyl linkages. The cross-linking procedure is simple, reproducible, and safe and does not employ any organic solvents. The result is a network of HA chains that is no longer water-soluble; hydrogels exhibit a mechanical behaviour typical of a strong gel and show favourable viscoelastic properties and an improved injectability profile [4,5].

Among its mechanical and rheologic properties, HA-DVS is characterized by a significant flexibility, ideal for a wide variety of medical applications, including post-surgical antiadhesive films, ocular fillings, and joint lubricants.

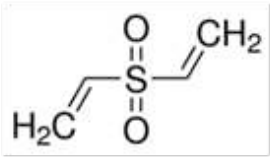
The relationship between molecular structure and cytocompatibility of HA-DVS of varying concentrations (0-100mM) has been recently investigated.

It has been showed that with increasing DVS concentration, the sulphur content and sulfonyl-bis-ethyl cross-link amount are increased and the mechanical stability and resistance against enzymatic degradation are enhanced.

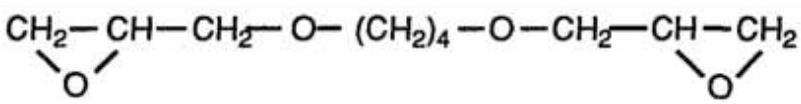
Cell viability, pro-inflammatory gene and cytokine expression, and glutamate uptake have been considered as valuable parameters to determine cytotoxicity: they are strongly dependent on the cross-linker concentration [4]. Furthermore, HA-DVS retains the biocompatibility and physical functionality of unmodified HA [5].

1,4-Butanediol diglycidyl ether is another cross-linking agent used to stabilize most the HA-based dermal fillers currently available on the market. Its cross-linking ability is attributed to the reactivity of the epoxide groups at the two ends.

DIVINIL SULFONE



ETERE 1,4 BUTANEDIOL DIGLICIDICO



Under basic (pH >7) conditions, the epoxide groups preferentially react with the most accessible primary alcohol in the HA backbone, thus producing an ether bond connection.

After reaction with HA, the epoxide groups of BDDE are neutralized, and only trace amounts of unreacted BDDE remain in the product (<2 ppm).

These trace amounts, which the FDA has determined to be below the level that is safe after a safety risk assessment, are prone to hydrolysis that ultimately yields CO2 and water.

Cross-linked HA is expected to follow a degradation pathway that is like that of un-cross-linked HA and unreacted BDDE, because cross-linking does not affect the HA backbone [6].

Physical properties
of HA fillers

Both DVS-HA and BDDE-HA are extremely safe product and can be used without any risk for patients. However, it is important to choose the right filler for the right patient to achieve the best cosmetic result.
To do this, it is of outstanding importance to understand physical properties of HA fillers and know how they behaviour in the skin to obtain the expected clinical outcome [7].

Among parameters that should be kept in mind to correctly evaluate and choose a filler, density and viscosity are essential.

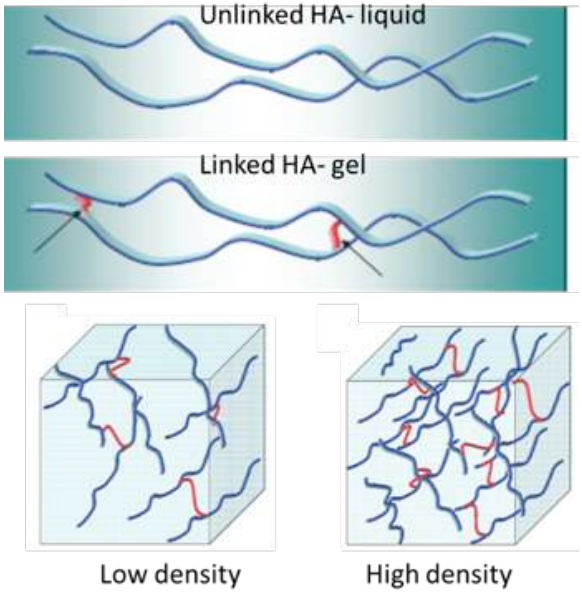
The cross-link density of a gel increases, when the distance between the cross-linked segments becomes shorter. When a load is applied, these shorter segments require a greater force to deflect. Thus, increasing cross-link density strengthens the overall network, thereby increasing the hardness or stiffness of the gel.

The elastic modulus G' is most often used to characterize the firmness of a gel.

Because G' describes the interaction between elasticity and strength, it provides a quantitative method for characterizing the hardness or softness of a gel [8].

- Gels with higher G' (higher stiffness) have a better ability to resist dynamic forces occurring during facial muscle movement and may provide better support and lift and longer duration of correction in areas such as nasolabial folds and marionette lines.

- Gels with low G' are likely better suited to areas with static and superficial wrinkles, where resistance to deformation is not critical, or areas where anatomy does not require stiffness but volume and softness are important, such as in lips.



The gel particles must be appropriately sized to be able to pass through these fine-bore needles with an acceptable extrusion force.

Firm gels, with a high ability to resist deformation, must be sized to small particles and should have a narrow distribution range to be easily injected through a thin-bore needle.

On the other hand, soft gels with low G' can have a broader distribution of particle sizes because the softer particles can be easily deformed to pass through the needle.

Regardless of whether a gel is firm or soft, particle size uniformity is preferred to avoid ''stop and go'' action during injections and for better control of gel placement [8].

The molecular weight is often reported on HA preparation. It is proportional to the number of repeating disaccharides in the HA molecule and it usually ranges from 500 to 6000 KDa.

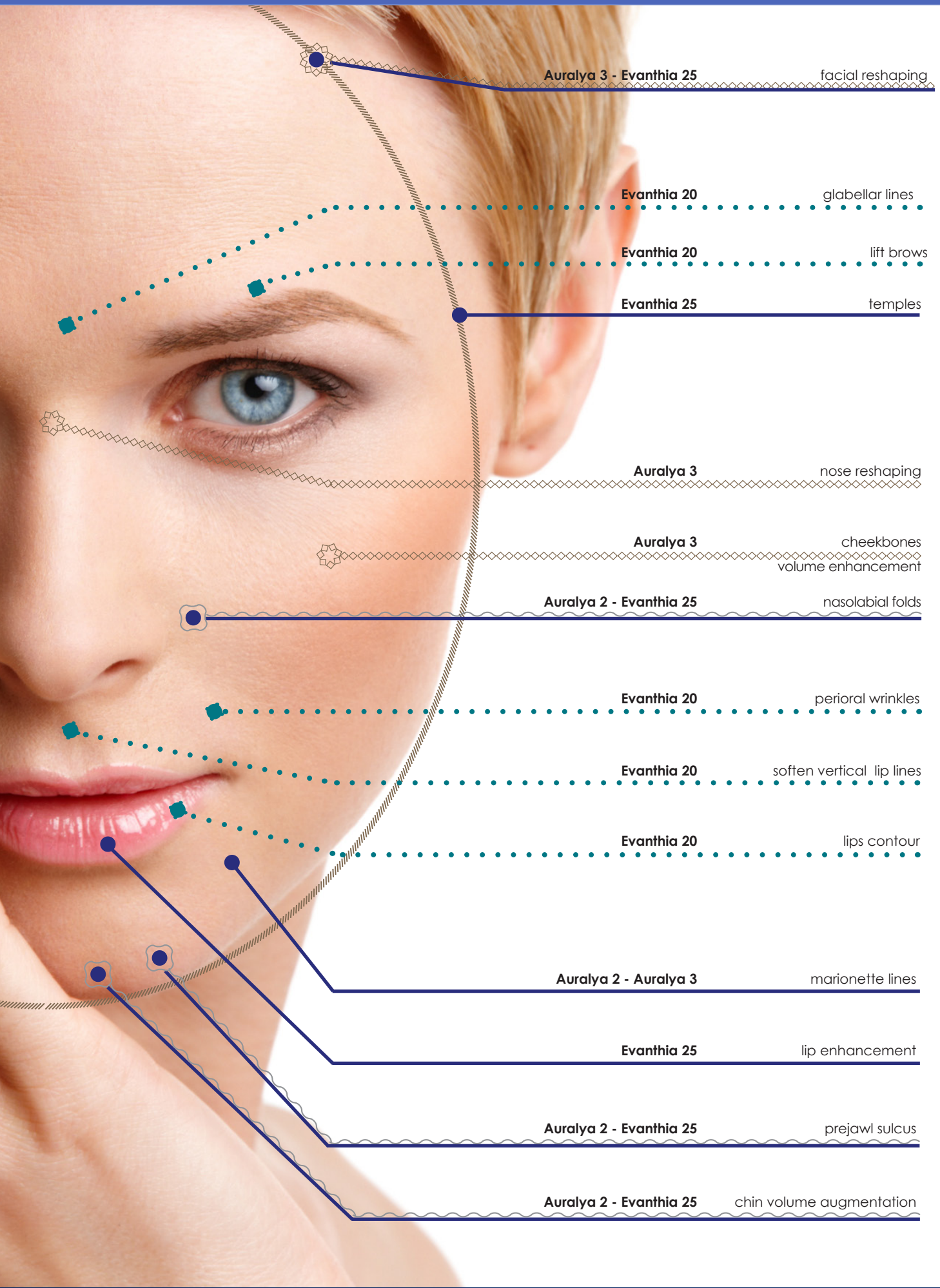
However, because the molecular weight of the final HA gel is enormous, small differences of the starting HA have little effect on the final properties of the gel [8].

Conclusion

A perfect hydrogel must have mechanical properties which simulate those of the ECM of natural tissues, sustain enzymatic degradation and withstand the compressive forces from the surrounding tissues in vivo, without deformation or collapse. It must possess superior injectability to pass through the pharmaceutical needles.

This feature is strictly dependent on cross-linking degree: a low cross-linking determines a soft gel with a good injectability but low stability in vivo; a high cross-linking degree results in an increase of the gel hardness and a less easy injectability profile, but good mechanical properties and an increased residence time.

All products currently available on the market meet strict safety requirements; therefore, the choice of a filler should be based on patient's characteristics, physical properties of hydrogels, and medical experience: there is no universal filler that is appropriate for every application or for every patient!


Auralya 3 - Evanthia 25
facial reshaping
Evanthia 20
glabellar lines
Evanthia 20
lift brows
Evanthia 25
temples
Auralya 3
nose reshaping
Auralya 3
cheekbones
volume enhancement
Auralya 2 - Evanthia 25
nasolabial folds
Evanthia 20
perioral wrinkles
Evanthia 20
soften vertical lip lines
Evanthia 20
lips contour
Auralya 2 - Auralya 3
marionette lines
Evanthia 25
lip enhancement
Auralya 2 - Evanthia 25
prejawl sulcus
Auralya 2 - Evanthia 25
chin volume augmentation

References

1. Alberts B, Johnson A, Lewis J, et al., editors. The molecular biology of the cell Cell junctions, cell adhesion, and the extracellular matrix 1065. New York: Garland Science; 2002.

2. Volpi N, Schiller J, Stern R, et al. Role, metabolism, chemical modifications and applications of hyaluronan. Curr Med Chem 2009;16(14):1718–1745.

3. Kogan G, Soltes L, Stern S, et al. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. Biotech Lett 2007;29:17–25.

4. Lai JY. Relationship between structure and cytocompatibility of divinyl sulfone cross-linked hyaluronic acid. Carbohydr Polym. 2014 30;101:203-12.

5. Milas M, Rinaudo M, Roure I, et al. Comparative rheological behavior of hyaluronan from bacterial and animal sources with cross-linked hyaluronan (hylan) in aqueous solution. Biopolymers 2001;59:191.

6. Be Buolle K, Glogau R, Kono T, et al. A Review of the Metabolism of 1,4-Butanediol Diglycidyl Ether–Crosslinked Hyaluronic Acid Dermal Fillers. Dermatol Surg 2013;39:1758–1766.

7. Borzacchiello A, Russo L, Malle B, et al. Hyaluronic acid based hydrogels for regenerative medicine applications. Biomed Res Inter 2015.

8. Kablik J, Monheit G, Yu L, et al. Comparative Physical Properties of Hyaluronic Acid Dermal Fillers Dermatol Surg 2009;35:302–312.

BIOFORMULA
ADVANCED BIOLOGICAL RESEARCH
The Italian Beauty Solution