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Should Hyaluronic Acid Fillers Be Diluted?

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At a recent American Society of Dermatologic Surgery one controversy arose between different experts on whether dilution of hyaluronic acid (HA) fillers is beneficial for the overall clinical efficacy.

HA is a negatively charged, linear, nonsulfated glycosaminoglycan consisting of repeating disaccharide units of glucuronic acid and *N*-acetylglucosamine.[1-3] HA is synthesized by three types of cell specific HA synthases (HAS1, HAS2, and HAS3) that are located in the cell membrane not the Golgi as other glycosaminoglycans, and regulated differentially in response to extracellular mediators.[2,3] With extrusion into the extracellular matrix (ECM), HA has an in vivo half-life of from hours to 2–3 days, depending on the types of tissues.[2,3] HA reacts with oxygen species or hyaluronidase, and is degraded in lysosomes or transferred into the circulation and cleared by the liver, lymph nodes or kidney.[2] Under normal conditions there is a tightly regulated equilibrium between the synthesis of HA and its turnover.[2]

HA can absorb large amounts of water due to its negative charges, and expands up to 1000 times in volume, forming a loose hydrated network.[1,2] Thus, HA acts as a space filler and can provide mechanical support and viscoelasticity in the ECM, as well as functioning as a lubricant, and osmotic buffer.[1,2] Hydrated HA networks control the transport of water and restricting the movement of pathogens, plasma proteins, and proteases.[1,2]

Covalent crosslinking is necessary to impart stability and can be used to modulate the functional properties of HA.[3] HA can be directly crosslinked without any chemical modifications, and has been crosslinked by bisepoxide or divinyl sulfone derivatives under alkaline conditions.[3] HA can also be crosslinked by glutaraldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), biscarbodiimide and multifunctional hydrazides under acidic conditions.[3] Compared to the native HA, the crosslinked hydrogels exhibit more robust mechanical properties and are less susceptible to enzymatic degradation.[3-5] Well-defined crosslinking chemistries have successfully introduced nanoscale and microscopic features to the existing HA bulk gels.[3]

If HA crosslinking takes place in a microscopic reaction vessel, HA hydrogel particles (HGPs, microgels or nanogels) can be produced.[3-5] HGPs exhibit definable size, large surface area, abundant interior space and addressable functional groups. HGPs are resistant to hyaluronidase digestion because the hyaluronidase in most cases cannot enter the HGPs, but HGPs remain sensitive to digestion by oxygen species.[3] Hydrogel matrices embedded with HGPs of micro- to nano- dimensions can provide tailored viscoelasticity and structural integrity, and have been used as tissue engineering scaffolds.[3]

However, the ability to oppose deformation and flattening secondary to natural elasticity or tension of the skin of different HA fillers (lift capacity) is considered to be a function not only of the elastic modulus (gel hardness or linear viscosity(G')), but also gel cohesivity.[1] Thus, cohesive HA gel fillers with a lower G' have been shown to have greater resistance to deformation than HGPs with a higher G' in linear compression tests.[1]

Dilution of HA fillers, which readily absorb water, will disturb the G' of HA fillers, particularly those which depend highly on HGPs for achievement of their G'.[1] For cohesive gel fillers, which depend on a high level of crosslinking, decreases in lift capacity with dilution should be less.[1] Other variables that may modulate the effects of dilution include the amount of water absorbed into the surrounding tissue, which would be less when injection are place into a relatively closed space.

The overall clinical effects and benefits of HA fillers, however, is not limited to the local lift capacity, and it has been shown that HA can induce new collagen formation.[6] With proper design, HA fillers can provide cells with a biologically relevant microenvironment that potentiates cell proliferation, migration, and ECM production.[6] Fibroblast-myofibroblast differentiation is associated with accumulation of a hyaluronan (HA) pericellular coat.[7,8] High molecular weight hyaluronic acid (HMWHA) is found in normal healthy tissue.[7,8] In injured tissue, HMWHA breaks down to low molecular weight HA (LMWHA), however, there are variations in the use of the terms HMWHA or LMWHA. HMWHA in general refers to any hyaluronic acid that has not been degraded.[7]

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Cells appear to be able to sense the difference between HMWHA, LMWHA, and oligo-HA.[7] HMWHA and LMHWA bind to CD44, TLR2, TLR4, LYVE, and RHAMM (CD168) receptors to accomplish their biological effects.[7,8] HMWHA is anti-inflammatory and antiangiogenic, and associates with and surrounds fibrobasts-myofibroblasts promoting differentiation and inducing collagen I and III production.[7,8] LMWHA binds to either TLR2 or TLR4 to elicit pro-inflammatory action, while HMWHA dampens inflammation by inhibiting TLR2 or TLR4 signaling.[7,8]

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LMWHA but not HMWHA stimulates macrophages to secrete inflammatory cytokines such as IL-8, while the maturation and activation of monocyte-derived dendritic cells is promoted by 1.2×10^3 Da HA, but not HMWHA or LMWHA.[7,8]

Hyaluronidase-treated HMWHA inhibited fibrocyte differentiation, while anti-CD44 antibodies potentiate fibrocyte differentiation, and CD44 appears to be the dominant receptor for HMWHA induced regulation of fibroblast-myofibroblast differentiation and collagen production.[7-9] Assembly and retention of the HA pericellular collagen matrix is dependent on hyaluronan-CD44 interactions.[9] The assembly of a pericellular HA coat and acquisition of the myofibroblastic phenotype is associated with re-localization of CD44 from a punctate distribution to a more diffuse staining pattern, and is an important regulator of the response of the cells to TGF-β1-driven fibroblast-myofibroblast transition.[9,10] There has been shown to be an age-related defect in synthesis of hyaluronan (HA) synthase (HAS) 2, which lead to an age-related defect in pericellular HA coat assembly with an associated decrease in dermal collagen and prolonged wound healing.[10]

There probably is a decrease in the G' and lift capacity with HA filler dilutions, particularly in HGP dominant fillers. However, that decrease may vary depending on the site of injection ie, relatively closed space within subcutaneous fat compartments vs the dermis. Dilution would increase diffusion of HGPs fillers more particularly in an open space than the cohesive gel fillers, again because of the differences in the physical characteristic of these product.[1] This might also suggest that dilution, if it did negatively affect duration of the HA, that effect would be more marked on HGPs. However, there may also not be a linear relationship to volume used to dilute the product. It is possible that with dilution of HA fillers, particularly cohesive gels, that these HA filler could distribute more easily around stromal cells, which could to induce fibroblast-myofibroblast differentiation and collagen production through CD44 binding. The later effect may be particularly beneficial in tissues such as the galea space or subcutaneous fat septae where mesenchymal stem cells are known to occur, and may contribute to the clinical efficacy of these agents.

There is evidence that HA fillers can induce collagen matrix production, and it is also known that HA can bind to receptors on fibroblasts to do this. The value of this has not been quantified for any of the products; however, the physical properties of the cohesive gel HA fillers do suggest that they would better adapt to dilution than HGP fillers. The amount of dilution which would optimize this potential effect on collagen production versus lift of the product is not known and may vary with the site of injection, but should be studied by the manufactures of these productions.

Disclosure

The author has not declared any relevant conflict of interest.

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