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Posted Date: 15 March 2024

doi: 10.20944/preprints202403.0883.v1

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Review

Current Knowledge and Regulatory Framework on the Use of Hyaluronic Acid for Aesthetic Injectable Skin Rejuvenation Treatments

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Abstract: Dermal injections of hyaluronic acid gel for aesthetic skin rejuvenation are increasingly popular nowadays. Although these products are classified as medical devices, the regulations on their administration by licensed practitioners are still weak, whereas their manufacturers increasingly highlight and advertise the cellular effects that underpin the efficacy of these injections. In this review, we discuss all current knowledge on the mode of action of dermally injected hyaluronic acid and the potential toxicological implications especially from crosslinked gels in conjunction with the current global regulations. We conclude that dermal injections of hyaluronic acid have several therapeutic implications that warrant further research, and that strict regulations must be applied on their manufacture/quality control and the required qualifications of licensed aesthetic injectors.

Keywords: dermal fillers; skin rejuvenation; collagen; hydrogel; hyaluronic acid; crosslinking; medical device; regulatory framework

1. Introduction

Hyaluronic acid (also known as Hyaluronan) is a versatile material with medical, pharmaceutical, and cosmetic applications due to its biocompatibility, biodegradability, and wide range of molecular weights [1–4]. It is a natural occurring linear anionic polysaccharide which consists of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked by β-1-3 and β-1-4 glycosidic covalent bonds. It is found in various tissues and biological fluids in the form of a sodium salt which is referred to as sodium hyaluronate [5]. It is synthesized in the plasma membrane by the hyaluronic acid synthase enzymes, then released into the extracellular matrix (ECM). In the human body, hyaluronic acid exists having molecular weights between 8.10^6 to 8.10^8 Da; an average human has 15g of hyaluronan, 1/3 of which is naturally degraded by hyaluronidase enzymes and synthesized by the synthase enzymes every day [6]. The degree of degradation is different for every person and can be affected by the exposure to environmental aggressors the person endures. For example, exogenous factors that can cause further degradation and faulted production of hyaluronan in the skin, are UV exposure and cigarette smoking; excessive UVB exposure causes the skin to become inflamed and the cells within the dermis to start producing less hyaluronan, whereas cigarette smoke was found to degrade hyaluronic acid *in vitro* [7–13].

Within the skin, hyaluronic acid in molecular weights between 4×10^6 Da – 6×10^6 Da is found in high concentrations in the basal layer of the epidermis, which is where proliferating keratinocytes are located [14]. The main function of hyaluronic acid in the epidermis is to maintain the extracellular structure that supports the extracellular matrix components (mostly fibroblasts) as well as providing an open, well hydrated structure for the passage of nutrients. An important property of hyaluronic acid is its ability to bind and retain water, therefore trapping moisture in the skin helping keep the skin hydrated. The acidic functional groups in the structure of hyaluronic acid hydrogels ionize at physiological pH; the negatively charged carboxylate anions repel each other, forcing the polymer

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network to expand. Water molecules are attracted to these charged groups and are then held on/bound to the polymer structure.

CD44 is a cell-surface glycoprotein receptor on fibroblasts that binds to hyaluronic acid in the basal layer of the skin [15]. The interaction between CD44 receptor and hyaluronic acid signals intracellular pathways that mediate several of the functions of hyaluronic acid in the skin, such as the synthesis of new collagen [16] and other homeostatic functions [14,17–20]. Other studies have suggested that the free-radical scavenging nature of hyaluronic acid contributes to protection against UVB and UVA radiation which supports the role of CD44 as a hyaluronic acid receptor in the epidermis [10,11]. The current popularity of hyaluronic acid as an ingredient in cosmetic products and in injectable aesthetic medical devices, alongside emerging research findings on its cellular effects, have instigated further studies [4,21–29].

The aim of our review is to discuss the cellular effects and toxicological implications of injecting hyaluronic acid into the skin for aesthetic purposes based on current knowledge, and the regulatory framework for these aesthetic medical devices.

2. The Role of Hyaluronic Acid on the Mechanical Properties of Young and Aging Skin

The mechanical and viscoelastic properties of the skin have been extensively covered in literature [30–32]. Young skin is plump and flexible; it is a "hydrated elastic solid" that can resist deformations and bounce back when stress (i.e., a mechanical force over a specific surface area) is applied to it; our facial skin is exposed to such forces daily because of our facial expressions such as frowning, smiling, raising our eyebrows. The firmness of youthful skin is attributed to the presence of a dense collagen and elastin protein network within the ECM of the dermis [33,34]. This dense protein network is communicating with the fibroblasts in the ECM via the hyaluronic acid chains which are also embedded within the ECM and keep it hydrated and provide structural support to the protein network. A healthy ECM and its components support the dermal-epidermal junction from separation and confer a cushioning effect against permanent skin surface deformations [34]. Studies have shown that between the ages of 20- and 60-years old, collagen and elastin degradation occurs via a decrease in their diameter leading to softening of the dermal protein network and a decrease in its ability to recoil elastically from deformation [32]. Fibroblast cells can produce new collagen from mechanical stimulation mediated via hyaluronan throughout a person's lifespan whereas the same elastin fibers are present from birth, and they cannot be easily replenished if damaged [35,36]. The softening of the dermal protein network in aged skin interferes with the mechanobiological pathways that stimulate collagen production by the fibroblast cells and subsequently further weaken the ECM structure contributing to the continuation of the aging process by preventing the epidermal renewal process [32,37,38].

These alterations in the protein content of the ECM matrix which underpin its gradual structural softening have been studied using Raman spectroscopy [39]. It is believed that the upper (papillary) dermal area is more affected than the lower (reticular) dermal area. From an aesthetic point of view, the mechanical forces that can cause a permanent deformation (i.e. static wrinkles) are much lower on aged skin; which means that the same facial expressions can result in wrinkles (permanent deformations) as our skin ages. Aged skin therefore behaves like a soft plastic solid that is less resilient to the effects of facial expressions [40]. This has been evidenced from mechanical measurements where skin tension and skin elasticity for children was 21 N/mm^2 and 70 N/mm^2 respectively, and 17 N/mm^2, 60 N/mm^2 in elderly adults [41].

Interventions to reverse the mechanical weakness of aged skin can rely on the stimulation of collagen production from fibroblasts by replenishing the ECM structure with injectable hyaluronic acid gel. The replenishment of elastin fibers is not as easy and therefore preventative measures (such as use of SPF, dietary nutrients) are necessary to prolong their longevity [42–45].

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3. The Current Use of Hyaluronic Acid in Skin Rejuvenation Products

The function of hyaluronic acid in formulations depends on its molecular weight; high molecular weight hyaluronic acid is greater than 1 x 10^6 Da, low molecular weight hyaluronic acid ranges from 0.8 to 8 x 10^5 Da and oligo-hyaluronic acid is smaller than 6 x 10^3 Da [46].

Studies on the diffusion of hyaluronic acid into the skin using Raman spectroscopy confirmed that only the low molecular weight (20-300 kDa) grades of hyaluronic acid can pass through the stratum corneum whereas high molecular weight HA (1000-1400 kDa) stays on the skin surface [47]. A recent in vitro study from L'Oreal found that both low and high molecular weight hyaluronic acid could potentially accumulate within the upper layers of the stratum corneum [48] reference and the use of mass spectrometry imaging has also been found effective for the quantification of hyaluronic acid within the skin [49].

The inclusion of low molecular weight hyaluronic acid in cosmetic formulas has become a beauty trend driven by the positive narrative the media portray. It is classified as a humectant or hygroscopic ingredient because of its ability to retain water molecules and therefore to boost hydration levels into the skin. Like other humectant ingredients, such as glycerin, low molecular weight hyaluronic acid is being used in many leave-on skin care products e.g. moisturisers and serums. Several randomnised clinical trial studies have been conducted on the antiageing effect of topically applied hyaluronic acid; the results showed significant reduction in wrinkle depth and significant improvement in skin elasticy and hydration versus the placebo, from formulations containing low molecular weight hyluronic acid [50–52].

High molecular weight hyaluronic acid polymer chains can hold water on the surface of the skin therefore they are used as either hydrogel skin film vehicles of active pharmaceutical and cosmetic ingredients, or in complexion-modifying semisolid cosmetics such as "skin-smoothing" primers, which upon application on the skin surface fill-up fine lines and wrinkles therefore improving their appearance for a temporary flawless effect [53].

Considering the above, it is evident that topically applied hyaluronic acid has limitations if a long-lasting or a significant effect on skin rejuvenation is desired.

Hyaluronic acid gels injected directly into the upper skin layers (dermis or epidermis) can overcome these limitations by by-passing the stratum corneum and enabling the deposition of hyaluronic acid into the skin irrespective of hyaluronic acid's molecular weight.

Injected hyaluronic acid hydrogels have comparable properties to that of the extracellular matrix (ECM) as their aqueous environment can support cell proliferation, migration, and nutrient diffusion with minimal mechanical irritation to surrounding tissues [54,55]. The aim of these injectable medical devices is to increase the skin's resilience against the formation of wrinkles, and/or to disguise static deep wrinkles.

They are either:

- Type 1 hydrogels i.e., consisting of partially crosslinked hyaluronic acid gel -- also called Dermal Fillers, or
- Type 2 hydrogels i.e., consisting of uncrosslinked hyaluronic acid viscous solution -- also called "Skin Boosters" or "injectable moisturisers".

Type 1 hydrogels are water-swellable but water-insoluble covalently crosslinked polymeric networks. They swell and retain water (or biological fluid) without loss of the structural integrity of the gel and because of their elastic nature they are used for the manufacture of implants such as breast implants. Whereas Type 2 hydrogels are devoid of covalent crosslinks in their structure; their linear polymer chains can form H-bonded crosslinks conferring a temporary gel network which behaves like a viscous liquid. Breakdown of the H-bonds upon stirring, flow (shear-thinning behaviour) or upon temperature changes (thermoreversible gelation), enable the conversion from a gel back to a solution.

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3.1. Dermal Fillers

Crosslinked hyaluronic acid is used in the manufacture of fillers. It absorbs moisture within the skin and swells to a predetermined maximum volume, which can then fill up wrinkles. It behaves like a structured elastic solid and can confer shape and volume when it is injected into the skin. The higher the degree of crosslinking, the stiffer the filler gel is, and the more difficult is to flow in the needle; that is why the hyaluronic acid in fillers is always partially crosslinked and not completely crosslinked; the partial crosslinking enables a degree of liquid-like (viscous) behaviour that allows flow. Whereas a completely crosslinked gel would have no flow and it would behave like an elastic implant [56,57].

The crosslinks in the hydrogel's structure resist the natural enzymatic degradation of hyaluronic acid by skin enzymes, therefore volumizing effect can last for 6 - 9 months [58]. The higher the degree of crosslinking, the lower the rate of biodegradation after injection and the longer lasting the contouring or volumizing effects are. Accidental injection of excess filler, or a desired reversal of the aesthetic result, can be dealt with by the administration of medicinal hyaluronidase enzyme solution [59–62].

Due to its physical properties, injected crosslinked hyaluronic acid fills up areas in the ECM of the dermis that contain fragmented and weakened protein network. These injected "pockets" then apply mechanical forces within the matrix that induce mechanical stretching and stimulation of the fibroblasts and increased collagen gene expression [63]. The stimulation of collagen synthesis after injection of dermal fillers can help partially restore the function of the ECM components that are damaged in the aged/photoaged skin, and it can also be useful therapeutically for the treatment of atrophic skin conditions [64,65].

It's been observed that crosslinked hyaluronic acid induces the accumulation of thick, densely packed Collagen I bundles as early as 4 weeks post-injection and continuing for at least a year [66–68]. Apart from Collagen I, it is believed that Collagens III, IV and VII are also produced via biopathways involved in wound healing [69].

Most dermal fillers contain BDDE (butanediol diglycidyl ether) as the crosslinking agent with a declared residual BDDE of less than 0.1 ppm [69].

3.2. Skin Boosters

Skin boosters contain uncross-linked high molecular weight (200kDa – 1500 kDa) hyaluronic acid polymer dissolved in water at concentrations ranging from 20mg/ml - 25mg/ml. In contrast to dermal fillers, they are not elastic (solid-like) material and therefore they do not have volumizing or contouring effect. In fact, the uncross-linked hyaluronic acid gel is a viscous liquid (its viscosity depends on the concentration of hyaluronic acid in water) and flows easily via the needle into the dermal tissue where it provides an instant moisturizing and hydrating effect. Because it is uncross-linked, it can undergo enzymatic degradation to lower molecular weight fragments into the skin, which are useful to stimulate collagen synthesis [70,71]. This regenerating effect starts a couple of weeks after injection. A gradual increase in collagen synthesis in combination with the hyaluronic acid gel in the extracellular matrix, can diminish the depth of existing wrinkles and prevent the formation of new wrinkles by increasing skin elasticity and resilience against deformation.

There is a fine balance between the requirement for the fragmented hyaluronic acid that can induce collagen production, and the complete enzymatic degradation of the injected hyaluronic acid gel which would undermine the plumpness of the extracellular matrix. The presence of trehalose in certain skin booster formulas is intended to keep this balance by protecting the hyaluronic acid from complete enzymatic degradation in the skin; this synergy between hyaluronic acid and trehalose is therefore claimed to provide a prolonged dual effect of hydration and collagen synthesis [72,73].

Skin boosters have also attracted attention because of their ability to improve skin texture of acne lesions; there has been lots of anecdotal evidence from aesthetic clinics for such smoothening effect. A recent randomized controlled trial compared the effect of skin boosters versus dermal fillers on moderate-to-severe atrophic acne scars; it was found that the skin booster produced a significant

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improvement compared to the traditional filler [74,75]. This can be attributed to the anti-inflammatory effect of hyaluronic acid in the skin and it's one of its numerous off-label uses [76–79].

Studies on the combination of botulinum toxin type A in combination with either cross-linked (dermal fillers) or non-cross-linked hyaluronic acid (skin boosters) for the treatment of atrophic acne scars, showed a better improvement in skin texture with the skin booster injections [80].

4. Crosslinking of Hyaluronic Acid in Dermal Fillers

Hyaluronic acid can physically crosslink itself when in aqueous solution, this happens due to the formation of a temporary intramolecular and intermolecular extended hydrogen-bonded system. The monomer has axial non-polar hydrogen atoms as well as polar side chains which create the hydrophobic/hydrophilic properties of the molecule. Due to the monomers linking alternatively between β-1, 4 and β-1, 3 bonds, each monomer will have inverted hydrophobic/hydrophilic faces compared to the monomer that will be next in the chain [81]. This is what causes hyaluronic acid to have a ribbon like structure, and theoretically when the polymer is placed in a solvent intramolecular and intermolecular interactions will occur as previously stated. This happens due to the polymer becoming stiff in solvent due to the internal hydrogen bonds forcing the polymer to rearrange into a physical crosslinked system with the solvent which is what forms a weak tridimensional platform. Usually, a crosslinking is added to create chemical covalent bonding between the chains instead of just the weak physical crosslink hyaluronic acid can form on its own in solution.

The modifications of the hydroxyl groups can come from four different reaction types: oxidation, ester formation, ether formation, or hemiacetal formation. Some of the reagents used in these reactions are: 1,4-butanediol-diglycidyl ether (BDDE), poly (ethylene glycol) diglycidyl ether (PEGDE) and divinyl sulfone (DVS) [82–87]. BDDE and PEGDE are the most common crosslinking agents for the manufacture of hyaluronic acid dermal fillers. A recent in-vitro comparative study on the toxicity of BDDE and PEGDE on human dermal cells concluded that PEGDE is a safer crosslinker than BDDE in terms of its lower cytotoxicity, inflammatory responses, ROS and MMP levels [82]. In *in-vitro* toxicity study models, both crosslinkers have the potential to cause toxicity to dermal cell lines exposed to a large volume of hyaluronic acid filler, by exceeding the maximum safe limit for residual crosslinker which is 2ppm. However, one of the dermal filler manufacturers has reported that residual BDDE is removed by purification steps during the manufacturing process [84].

5. Toxicology and Current Safety Regulations on Approved Crosslinking Agents in Dermal Fillers

In accordance with global medical device regulations, specific standards published by the International Standardization Organisation (ISO) are put in place to further regulate processes and support the safety and efficacy at every stage of development of medical devices. ISO 10993 is a set of standards which consist of twenty distinct parts and is the ISO standard which specifically lays out the principles relating to the biocompatibility of medical devices. Some topics covered are biological evaluation of medical devices (Part 1), tests for in vitro cytotoxicity (part 5), tests for genotoxicity, carcinogenicity, and reproductive toxicity (part 3), and tests for local effects after implantation (part 6). The requirements state how medical devices undergo biological evaluation and risk management with general principles regarding evaluation of current relevant data, identification of gap analysis, and the evaluation of the biological safety of the device. Although this standard is currently in force, it is set to be replaced by an updated standard of the same name, however this is currently under development [88].

BDDE is the longest used crosslinking agent for HA, with biological data backing its use and efficacy. As a stand-alone raw material, the current toxicological profile of BDDE shows the potential of acute toxicity for oral and dermal exposure, with potential of skin irritation, eye damage, and harm where inhalation is the route of exposure [89]. PEGDE is a difunctional crosslinker for amine-, hydroxyl-, and carboxyl-functional polymers. As a stand-alone raw material, the current toxicological profile of PEDGE shows low toxicity, with potential for skin and eye irritation. DVS is a crosslinking agent sulfone compound which has two S-vinyl substituents. As a stand-alone raw material, the

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current toxicological profile of DVS shows a human toxicity with the ability to cause burns of the skin (dermatoxin) & eyes (lacrimator), as well as cause injury & enzyme inhibition by condensing with amino & other groups [90].

When looking at risk assessment and toxicological data it is important to consider the relevancy of the data based on the route and type of exposure of the medical device. For example, what is the theoretical level of exposure of the material in the final device and does this have potential to reach a level to cause assessed effects? Has the process of manufacturing eliminated most of the material? What is the route of delivery, and where is it intended to be delivered? Will the location of delivery accelerate the breakdown and absorption of the device? These are considerations which can help in the determination of how particularly toxic something will potentially be to the human body.

6. Lymph Node Blockage and Cancer Risk of Dermal Fillers

Recent studies and media coverage have shone a light on the potential of dermal fillers causing lymphatic obstruction, and in turn being the aggravator of diseases such as cancer [91]. Drainage of lymphatic fluid is a pivotal part of the human body's infection and disease control, with the lymph channels transporting pathogens in the lymphatic fluid to the lymph nodes to be contained and destroyed [92]. Blockage of the channels to the lymph nodes may cause lymphoedema (swelling), as well as potential delays in the activation of the immune response to pathogens which could theoretically increase the risk of infection and disease [93].

In the common day many areas of the face are subjected to dermal fillers, with injectables commonly being introduced to the lips and undereye. Draining of lymphatic fluid from the labium superius oris and labium inferius oris passes through the submandibular lymph nodes primarily, as well as lymphatic fluid from the medial of the labium inferius oris passing through the submental lymph node initially. The infraorbital lymph nodes are located beneath the eye [92].

Indeed, visual side effects caused by HA filler-induced embolization are common in tertiary medical care; a 5-year study found that intraarterial thrombolytic treatment (IATT) was successful in restoring visual acuity in 36% (26 out of 72) cases [94].

Such side effects are however avoidable and depend on the skill, experience, and knowledge of the injector. Crosslinked dermal fillers differ in their rheological properties and an expert aesthetic practitioner would be able to choose the most appropriate products for each anatomic location and desired cosmetic outcome [95–101].

The hypothesis on the risks of hyaluronic acid fillers specifically blocking lymphatic channels and causing cancer is still preliminary but has alerted the British Association of Aesthetic Plastic Surgeons (BAAPS) in planning further research and entering in discussions with government officials to tighten the regulations in the UK regarding the required qualifications of aesthetic practitioners and the products that can be used [91]. It can be assumed that regulatory health bodies are standing by to observe relevant findings before analyzing the need for imposing any potential further restrictions. Biophysical studies using Magnetic Resonance Imaging on the localization and longevity of hyaluronic acid fillers after injection into the facial dermis could help elucidate the claimed risks [102].

7. Global Regulatory Framework on Injectable Hyaluronic Acid Medical Devices

The importance of global harmonisation of regulations referring to dermal fillers is pivotal for the trust in confidence of consumer safety. Reviewing the global regulations, shows that the majority of injectable dermal fillers, no matter the composition of the filler itself, are classified as Class III medical devices due to their nature, how they are implanted, and ability to naturally (or mechanically) break down and absorb into the human body.

The EU Medical Device Regulation (Regulation (EU) 2017/745) defines dermal fillers as class III medical devices in accordance with Annex XVI [103]. Post Brexit, the UK regulate medical devices through the Medicine and Healthcare products Regulatory Agency (MHRA), how impose the Medical Device Directive 93/42/EEC, Active Implantable Medical Devices Directive 90/385/EEC, and the in-vitro Diagnostic Medical Devices Directive 98/79/EC. The Directives are not the current

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Directives in place in the EU market, but the same baseline considerations on classification of medical devices are in place which in turn puts dermal injections as a Class III Medical Device on the UK market [104]. Northern Ireland follows the current EU Medical Device Regulation and its additional Directives, as opposed to the slightly outdated versions used under UK regulation.

The US classifies absorbable dermal fillers as class III medical devices in accordance with The Code of Ferdal Regulations Title 21, Chapter I, Subchapter H on Medical Devices which stipulates that these fillers would be classified as "implants" in regulatory text as they are intended to remain within a cavity of the body for 30 days or more [105].

In China, invasive injectables of any kind are classified as Class III medical devices according to the Medical Devices Supervision and Administration Regulation (MDSAR) based on the longevity of the filler, the system of delivery (injection), and the fact that the dermal filler can be absorbed by the human body [106].

In Australia, dermal fillers are medical devices classified as Class III by the classification rules set out in Schedule 2 to the Therapeutic Goods (Medical Devices) Regulation 2002, falling out of the scope of the exception criteria [107]. Hyaluronic acid and its polymers are listed in Schedule 4 (Prescription only medicines and prescription animal remedies) of the Poison Standard allowing its use when used in preparation for injection or implantation [108].

8. Conclusions

Dermal injections of hyaluronic acid gel are not just an aesthetic temporary intervention; all current knowledge on the mode of action of hyaluronic acid in the skin, corroborates the pharmacological effects of these injections which underline their skin regenerative effects; these include instigation of collagen production via biomechanical stimulation of dermal fibroblasts, and anti-inflammatory pathways that alleviate acne and improve skin texture. Such therapeutic implications alongside the severity of the side effects from poor injection practices and the toxicological implications from the systemic absorption of residual crosslinking agents that might be present, reiterate the need for strict regulations on i) the manufacture and quality control of these medical devices, and ii) the qualifications and license acquisition of insured aesthetic injectors.

Author Contributions: Conceptualization, J.A. and K.D.; writing—original draft preparation, J.A. and K.D.; writing—review and editing, K.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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