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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 becoming 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 also highlight the urgent need for further research to elucidate the therapeutic implications and underscore the imperative need for robust regulatory frameworks to safeguard public health. We conclude that dermal injections of hyaluronic acid have several therapeutic implications that warrant further research and that strict regulations must be applied to 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

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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 naturally occurring linear anionic polysaccharide that 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 synthesised in the plasma membrane by the hyaluronic acid synthase enzymes and then released into the extracellular matrix (ECM). In the human body, hyaluronic acid exists with molecular weights between 8×10^6 and 8×10^8 Da; an average human has 15 g of hyaluronan, one third of which is naturally degraded by hyaluronidase enzymes and synthesised by the synthase enzymes everyday [6]. The degree of degradation is different for every person and can be affected by the exposure to environmental aggressors that the person experiences. For example, exogenous factors that can cause further degradation and the defective 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 [7–10], whereas cigarette smoke was found to degrade hyaluronic acid in vitro [11]. According to these studies, the presence of hyaluronic acid in the dermis provides a protective mechanism against UVB radiation [7,8], and evidence of this homeostatic photoprotective mechanism is the upregulation of hyalase synthase enzymes upon UVB exposure [9]. Furthermore, the role of plasma hyaluronic acid in lung function and health was evidenced by significantly

increased hyalase activity and subsequently decreased hyaluronic acid plasma levels in COPD patients [12].

Within the skin, hyaluronic acid in molecular weights of 4×10^6–6×10^6 Da is found in high concentrations in the basal layer of the epidermis, which is where the proliferating keratinocytes are located [13]. 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 and helping to keep the skin hydrated. The acidic functional groups in the structure of hyaluronic acid hydrogels ionise at physiological pH; the negatively charged carboxylate anions repel each other, forcing the polymer network to expand. Water molecules are attracted to these charged groups and are then held/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 [13]. The interaction between the 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 [14] and other homeostatic functions [13,15–19]. 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 [9,10]. 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, has instigated further studies [4,20–28].

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 in the Mechanical Properties of Young and Aging Skin

The mechanical and viscoelastic properties of the skin have been extensively covered in the literature [29–31]. Young skin is plump and flexible; it is a "hydrated elastic solid" that can resist deformation 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, or 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 [32,33]. This dense protein network communicates 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 protect the dermal–epidermal junction from separation and confer a cushioning effect against permanent skin surface deformation [33]. 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 the softening of the dermal protein network and a decrease in its ability to recoil elastically from deformation [31]. Fibroblast cells can produce new collagen throughout a person's lifespan through mechanical stimulation mediated via hyaluronan. In contrast, elastin fibres are present from birth, and they cannot be easily replenished if damaged [34,35]. 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 weakens the ECM structure, contributing to the continuation of the aging process by preventing the epidermal renewal process [31,36,37].

These alterations in the protein content of the ECM matrix that underpin its gradual structural softening have been studied using Raman spectroscopy [38]. 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 permanent deformation (e.g., 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 [39]. This has been evidenced by mechanical measurements, where the skin tension and skin elasticity for children were 21 N/mm^2 and 70 N/mm^2, respectively, and they were 17 N/mm^2 and 60 N/mm^2 in elderly adults [40].

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 fibres is not so easy and therefore preventative measures (such as the use of SPF, dietary nutrients) are necessary to prolong their longevity [41–44].

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 1×10^6 Da, low-molecular-weight hyaluronic acid ranges from 0.8 to 8×10^5 Da, and oligo-hyaluronic acid is smaller than 6×10^3 Da [45].

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 [46]. 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 [47], and the use of mass spectrometry imaging has also been found to be effective for the quantification of hyaluronic acid within the skin [48].

The inclusion of low-molecular-weight hyaluronic acid in cosmetic formulas has become a beauty trend driven by the positive narrative that the media portray. It is classified as a humectant or hygroscopic ingredient because of its ability to retain water molecules and therefore to boost the hydration levels in the skin. Like other humectant ingredients, such as glycerine, low-molecular-weight hyaluronic acid is being used in many leave-on skincare products, e.g., moisturisers and serums. Several randomised clinical trial studies have been conducted on the anti-aging effect of topically applied hyaluronic acid; the results show a significant reduction in wrinkle depth and significant improvements in skin elasticity and hydration versus the placebo, for formulations containing low-molecular-weight hyaluronic acid [49–51].

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 in fine lines and wrinkles, therefore improving their appearance for a temporary flawless effect [52].

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 bypassing the stratum corneum and enabling the deposition of hyaluronic acid into the skin irrespective of the hyaluronic acid's molecular weight.

Injected hyaluronic acid hydrogels have comparable properties to the extracellular matrix (ECM) as their aqueous environment can support cell proliferation, migration, and nutrient diffusion, with minimal mechanical irritation to surrounding tissues [53,54]. 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:

- Predominantly Type 1 hydrogels, i.e., consisting of a partially crosslinked hyaluronic acid gel, also called dermal fillers.
- Type 2 hydrogels, i.e., consisting of an 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 the 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. Meanwhile, type 2 hydrogels are devoid of covalent crosslinks in their structures; their linear polymer chains can form H-bonded crosslinks, conferring a temporary gel network, which behaves like a viscous liquid. The breakdown of the H-bonds upon stirring, flow (shear-thinning behaviour), or temperature changes (thermoreversible gelation) enables conversion from a gel back to a solution.

3.1. Dermal Fillers

Dermal fillers contain partially crosslinked hyaluronic acid aqueous gels. The crosslinked gel behaves like a structured elastic solid, absorbs additional moisture within the skin, and swells to a predetermined maximum volume; when carefully injected, it can improve the shape and volume/contour [55,56] and fill in wrinkles [39]. The higher the degree of crosslinking, the stiffer the filler gel is, and the less able it is to flow in the needle; therefore, the hyaluronic acid gel in fillers is always partially crosslinked; partial crosslinking enables a degree of liquid-like (viscous) behaviour that allows flow, in contrast to a completely crosslinked gel, which would have no flow and would behave like an elastic implant [39].

The crosslinks in the hydrogel's structure resist the natural enzymatic degradation of hyaluronic acid by skin enzymes; therefore, the volumizing effect can last for 6–9 months [57]. The higher the degree of crosslinking, the lower the rate of biodegradation after injection and the longer-lasting the contouring or volumizing effects are. The accidental injection of excess filler, or the desired reversal of the aesthetic result, can be dealt with by the administration of a medicinal hyaluronidase enzyme solution [58–61].

Due to its physical properties, injected crosslinked hyaluronic acid fills 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 the mechanical stretching and stimulation of the fibroblasts and increased collagen gene expression [62]. The stimulation of collagen synthesis after the injection of dermal fillers can help to partially restore the function of the ECM components that are damaged in aged/photoaged skin, and this can also be useful therapeutically for the treatment of atrophic skin conditions [63,64]. It has 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 [65–67]. Apart from collagen I, it is believed that collagens III, IV, and VII are also produced via biopathways involved in wound healing [68].

Most dermal fillers contain butanediol diglycidyl ether (BDDE) as the crosslinking agent, with declared residual BDDE content of less than 0.1 ppm [68]. Current research in the technology of fillers is aiming to expand their compositions or replace hyaluronic acid with alternative gelling agents. A recent clinical trial on eyebrow augmentation using fillers enriched with the amino acids glycine and proline reported a positive effect [69]. Another study aimed to establish the rheological properties of such dermal filler formulations containing additives [70].

The rationale for the addition of these amino acids was not explained in the study but we presume that they were added for their collagen-production-inducing effect [71]. There was no control group in this study; therefore, we cannot assume a significant benefit compared to the current commercial dermal fillers. It is necessary that studies are carried out considering whether it is justifiable to increase the complexity of the dermal fillers' compositions.

Agarose gel was studied for its efficacy as a dermal filler, with positive clinical outcomes in terms of facial contouring and patient satisfaction [72]. However, more clinical and safety data are required that compare its use in aesthetic dermal fillers instead of hyaluronic acid.

3.2. Skin Boosters

Skin boosters contain an uncrosslinked high-molecular-weight (200–1500 kDa) hyaluronic acid polymer dissolved in water at concentrations ranging from 20 mg/mL to 25 mg/mL. In contrast to dermal fillers, they are viscous (liquid-like) materials and they do not have volumizing or contouring effects [39]. The uncrosslinked hyaluronic acid gel is a viscous liquid (its viscosity depends on the concentration of the hyaluronic acid polymer in the aqueous solvent) and it flows easily from the needle into the dermal tissue, where it provides an instant moisturising and hydrating effect [39]. Because it is uncrosslinked, it can undergo enzymatic degradation by hyalase enzymes in the dermis at a quicker rate than the crosslinked hyaluronic acid gel in dermal fillers. The enzymatic degradation of hyaluronic acid to lower-molecular-weight fragments is instrumental to the stimulation of collagen synthesis in the dermis [39,73,74]. This regenerating effect starts a couple of weeks after the injection of the skin booster; the 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 the skin's elasticity and resilience against deformation [39].

There is a fine balance between the requirement for the enzymatically fragmented low-molecular-weight hyaluronic acid, which can induce collagen production in the dermis, and the complete enzymatic degradation of the injected gel, which would diminish its boosting effect on the plumpness of the extracellular matrix [39]. The presence of trehalose in certain skin booster formulas is intended to ensure this balance by protecting the hyaluronic acid from complete enzymatic degradation in the skin and therefore prolonging the presence of high-molecular-weight hyaluronic acid. This synergy between hyaluronic acid and trehalose is claimed to provide a prolonged dual effect of hydration and collagen synthesis [39,75,76]; we presume that the latter effect would occur via the prolonged biomechanical stimulatory effect of the viscous gel within the dermis.

Skin boosters have also attracted attention because of their ability to improve the texture of acne lesions; there has been a great deal of anecdotal evidence from aesthetic clinics for such smoothing effects. A recent randomised controlled trial compared the effects of skin boosters versus dermal fillers on moderate-to-severe atrophic acne scars; it was found that the skin booster produced a significant improvement compared to the traditional filler [77,78]. This can be attributed to the anti-inflammatory effect of hyaluronic acid in the skin, and it is one of its numerous off-label uses [79–82].

Studies on the combination of botulinum toxin type A in combination with either crosslinked (dermal fillers) or non-crosslinked hyaluronic acid (skin boosters) for the treatment of atrophic acne scars showed a better improvement in skin texture with skin booster injections [83].

4. The Crosslinking of Hyaluronic Acid in Dermal Fillers

Hyaluronic acid can physically crosslink itself when in an 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 lead to 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 is next in the chain [84]. 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 the solvent due to the internal hydrogen bonds, forcing the polymer to rearrange into a physical crosslinked system with the solvent, which forms a weak tridimensional platform. Usually, a crosslinker is added to create chemical covalent bonding between the chains, instead of merely the weak physical crosslink that hyaluronic acid can form on its own in a 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) [85–90]. 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 [85]. In in vitro toxicity study models, both crosslinkers have shown 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 2 ppm. However, one of the dermal filler manufacturers has reported that the residual BDDE is removed by purification steps during the manufacturing process [87].

5. The 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 of medical devices at every stage of development. ISO 10993 is a set of standards that consists of twenty distinct parts and is the ISO standard that specifically lays out the principles relating to the biocompatibility of medical devices [91]. Some topics covered are the 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 the evaluation of current relevant data, the identification of gap analysis, and the evaluation of the biological safety of the device. While this standard is currently in force, it is set to be replaced by an updated standard of the same name; however, the updated standard is currently under development [91].

BDDE is the longest used crosslinking agent for HA, with biological data backing its use and efficacy. As a standalone raw material, the current toxicological profile of BDDE shows the potential for acute toxicity for oral and dermal exposure, with the potential for skin irritation, eye damage, and harm when inhalation is the route of exposure [92]. PEGDE is a difunctional crosslinker for amine-, hydroxyl-, and carboxyl-functional polymers. As a standalone raw material, the current toxicological profile of PEDGE shows low toxicity, with potential for skin and eye irritation. DVS is a crosslinking agent and sulfone compound that has two S-vinyl substituents. As a standalone raw material, the current toxicological profile of DVS shows human toxicity, with the ability to cause burns of the skin (dermatoxin) and eyes (lacrimator), as well as causing injury and enzyme inhibition by condensing with amino and other groups [93].

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 the potential to reach a level to cause the 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 that can help in the determination of how toxic an item will potentially be to the human body.

6. The Lymph Node Blockage and Cancer Risks of Dermal Fillers

Recent studies and media coverage have shed light on the potential of dermal fillers to cause lymphatic obstruction and in turn to aggravate diseases such as cancer [94]. The 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 [95]. 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 [96].

At present, many areas of the face are subjected to dermal fillers, with injectables commonly being introduced to the lips and undereye area. The drainage of lymphatic fluid from the labium superius oris and labium inferius oris passes through the submandibular lymph nodes primarily, and lymphatic fluid from the medial of the labium inferius oris passes through the submental lymph node initially. The infraorbital lymph nodes are located beneath the eye [95].

Indeed, visual side effects caused by HA-filler-induced embolisation 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) of cases [97].

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 product for each anatomic location and desired cosmetic outcome [98–104].

The hypothesis regarding the risks of hyaluronic acid fillers in specifically blocking the lymphatic channels and causing cancer is still preliminary. However, it has triggered the British Association of Aesthetic Plastic Surgeons (BAAPS) to plan further research and engage in discussions with government officials to tighten the regulations in the UK concerning the required qualifications of aesthetic practitioners and the products that can be used [94].

It can be assumed that regulatory health bodies are standing by to observe relevant findings before analysing the need to impose any potential further restrictions. Biophysical studies using magnetic resonance imaging on the localisation and longevity of hyaluronic acid fillers after injection into the facial dermis could help to elucidate the claimed risks [105].

7. The Global Regulatory Framework on Injectable Hyaluronic Acid Medical Devices

The importance of the global harmonisation of regulations referring to dermal fillers is pivotal for confidence in consumer safety. A review of the global regulations shows that the majority of injectable dermal fillers, regardless of the composition of the filler itself, are classified as class III medical devices due to their nature, how they are implanted, and their 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 [106]. Post Brexit, the UK regulates medical devices through the Medicine and Healthcare Products Regulatory Agency (MHRA), which imposes 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. These directives are not the current directives in place in the EU market, but the same baseline considerations for the classification of medical devices are in place, which in turn classifies dermal injections as a class III medical procedure in the UK market [107]. 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 should be classified as “implants” in regulatory text as they are intended to remain within a cavity of the body for 30 days or more [108].

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 [109].

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 beyond the scope of the exception criteria [110]. Hyaluronic acid and its polymers are listed in Schedule 4 (Prescription only medicines and prescription animal remedies) of the Poison Standard, allowing their use when applied in preparation for injection or implantation [111].

8. Conclusions

Dermal injections of hyaluronic acid gel are not merely 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 the instigation of collagen production via the biomechanical stimulation of dermal fibroblasts and anti-inflammatory pathways that alleviate acne and improve the skin texture. Such therapeutic implications, alongside the severity of the side effects due to poor injection practices and the toxicological implications of the systemic absorption of the 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.

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