

The Potential Use of Novel Plant-Derived Recombinant Human Collagen in Aesthetic Medicine

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Summary: Recombinant human type I collagen, identical in structure and functionality to human type I collagen, was successfully expressed and extracted from genetically modified tobacco plants. Contrarily to tissue extracted protein, rhCollagen is not immunogenic and not allergenic and has an intact triple helix structure showing superior biological functionality. A photocurable rhCollagen was developed by chemically modifying the protein to allow cross-linking under illumination at various wavelengths, maintaining the protein structural and biological functions. The use of the photocurable rhCollagen in aesthetic medicine, especially as a dermal filler and as a bioink for 3D-printed breast implant is discussed in this article. (*Plast. Reconstr. Surg.* 148: 32S, 2021.)

Collagen, the predominant protein of human extracellular matrix, being about 30% of human dry weight, confers physical support, high-tensile strength, and resilience to tissues and organs.^{1–5} In addition to the structural role, collagen interacts with a variety of macromolecules such as integrins, decorin, fibronectin, heparin, and matrix metalloproteinases (MMPs)⁶ regulating essential steps in the tissue regeneration process.

Thus far nearly 29 types of collagen have been identified,⁷ within them the most common are type I, found in skin, tendon, and bone, type II found in cartilage, and type III found in skin and vasculature.⁸ Collagen type I, constituting the 80%–90% of all collagen types⁹ is an heterotrimer composed of two alpha 1 and one alpha 2 chains each composed of repeating Gly-X-Y triplets, where X and Y can represent any amino acid but are typically proline and hydroxyproline.^{1,3,4,9}

Having major structural and biological functions, collagen has been considered one of the most promising biomaterial in biomedical and aesthetic medicine applications. However, the most common sources for these applications are animal and, in some cases, human cadavers

where collagen is already fibrillated, cross-linked, and embedded into the native tissues. Moreover, during the extraction and purification process, the material undergoes a series of harsh purification steps that cause irreversible modifications damaging its biofunctionality and stability. In addition, being extracted from animal or human or tissues, risks of pathogen contaminations and allergenicity are not negligible.^{5,10–12} Allergy tests are in fact required prior injecting bovine extracted collagen products in biomedical or aesthetic applications.¹³

Inspired by the important role of collagen in tissue function, matrix remodeling, tissue repair, and regeneration, a tobacco plant expression platform technology was developed at the Hebrew University in Israel to effectively express human type I collagen.^{5,10,11,14} The highly complex and hierarchical posttranslational process of collagen is a prerequisite of full maturation and functionality of the protein and its fibrillar structure. Through a series of genetic transformations followed by extensive genetic and

From CollPlant Ltd.

Received for publication June 4, 2021; accepted September 22, 2021.

Presented in part at the 18th Aesthetic & Anti-Aging Medicine World Congress, November 5 through 7, 2020.

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DOI: 10.1097/PRS.00000000000008784

Disclosure: All authors are employees of CollPlant Ltd. As of February 2021, CollPlant is part of a worldwide exclusive development and commercialization agreement for dermal and soft-tissue filler products with Allergan Aesthetics, an AbbVie Company.

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phenotypic selections, five synthetic genes encoding the relevant human proteins were successfully co-expressed in tobacco plant vacuoles.^{10,14} These include two synthetic genes encoding the human heterotrimeric type I collagen (COL1 α 1 and COL1 α 2) and three genes encoding human modifying enzymes critical to collagen maturation and posttranslational modification (P4H-a P4H-b and LH3). The plant extracted recombinant human type I collagen (rhCollagen) shows a thermally stable triple helical structure, fibrillates and demonstrates bioactivity resembling that of native collagen.¹⁰ rhCollagen is identical to the naive newly synthesized human type I collagen, its structure is intact and it is fully functional.

The plant extracted rhCollagen has also shown zero immune response in a sensitive assay with human CD4+ T-cells, whereas the bovine extracted collagen control had the expected higher response in this assay.¹⁵ This result confirms that there is no potential risk of immune response when using rhCollagen in a clinical setup.¹⁵

The purified rhCollagen was in fact used as a main component of medical products commercially available in EU in the field of wound healing and orthobiologics^{16–18} with excellent efficacy and safety performance.

In this article, we review the new frontiers opened by the plant-derived recombinant human type I collagen in the field of regenerative medicine, focusing in aesthetic medicine, tissue rejuvenation, and 3D bioprinting.

PHOTOCURABLE RECOMBINANT HUMAN TYPE I COLLAGEN

Leveraging the properties of the plant-derived rhCollagen, such as biofunctionality, homogeneity, purity, and safety, rhCollagen was chemically modified with methacrylic groups to achieve a photocurable monomeric collagen-methacrylamide. The methacrylic groups are linked to the core collagen molecule via a zero distance functionalization (rhCollagen-MA), or via a polyethylene glycol (PEG) linker (rhCollagen-PMA). rhCollagen-MA and rhCollagen-PMA maintain the physical and biological properties of its collagen source while allowing cross-linking when illuminated at various wavelengths using different photoinitiators. Rheological and mechanical properties of the modified rhCollagen were characterized, and it was successfully included in formulations in a variety of applications with and without cells, from dermal filler to 3D organ prototypes printing.

RECOMBINANT HUMAN COLLAGEN AND COLLAGEN-MA IN AESTHETIC MEDICINE

Photocurable Dermal Filler

Imbalanced collagen homeostasis is a known origin of skin aging. The reduction in collagen biosynthesis in concomitance with the increase in fibril fragmentation, results in net collagen deficiency inducing thinning of the skin and wrinkles appearance.¹⁹ With aging of the population and the increasing desire for youthful appearance, the demand for safe, minimally invasive, and effective soft-tissue augmentation procedures is constantly growing. More and more companies are on the search for safer and longer lasting fillers. Being the major responsible for the skin mechanical properties and tensile strength, bovine collagen was the first dermal filler to be approved by the Food and Drug Administration in 1981, becoming soon the “gold standard” among injectable fillers.^{13,20} The use of bovine-derived collagen was associated, however, with granulomas, and localized hypersensitivity reaction and has a certain risk of xenogeneic transmission of pathogens.¹³

In 2003, a revolution in the dermal filler market commenced when the first hyaluronic acid (HA)-based product was approved by the Food and Drug Administration turning in few year into the most popular injection filler for soft-tissue correction.²¹ HA is a linear polysaccharide composed of repeated disaccharide units of glucuronic acid and N-acetylglucosamine. It is found in high amounts in several connective tissues forming a substantial part of the extracellular matrix.^{22,23} In the skin, HA is present in a very high percentage and plays a crucial role in tissue hydration and volume preservation thanks to its viscoelasticity and water retention capacity.²⁴ In its linear form, HA is highly biocompatible, has an anti-inflammatory effect, and activates signaling pathways through the interaction with cell membrane receptors.^{25,26} Nevertheless, it degrades *in vivo* extremely rapidly, between hours to few days²³ making it impossible to be used in its native structure in aesthetic applications where the lifting effect should last for several months. The great majority of HA-based dermal filler products are indeed chemically cross-linked to enhance the mechanical stability and longevity of the injected material. Chemical cross-linking, however, modifies the natural structure of the molecule affecting its biological activity and cells affinity.²⁵ Hence, despite the promising properties of its original molecule, cross-linked HA dermal fillers do not significantly promote tissue

regeneration²⁶ but rather mechanical support and skin lifting thanks to its viscoelastic properties, as long as the injected material is not resorbed.²⁷

The lifting capacity of an injectable soft-tissue filler is proportional to its mechanical properties. The elasticity of a gel, quantified as the elastic (storage) modulus (G'), is a measure of the gel's ability to resist deformation when shear stress is applied.²⁸ The higher the G' of a gel, the less it deforms and the more stored energy is retained. Fillers with high G' better resist external forces and provide superior structure support.²⁸ Clinical objectives as lifting, support, and sculpting are thus better achieved using fillers with high values of G' .²⁸ In terms of mechanical properties, nowadays, dermal fillers are, however, limited by the inner diameter of the needle used. Fillers are injected in the majority of the cases into very delicate and sensitive areas of the face requiring needles as thin as 25–30G, depending on the area treated (Gel at 0.7 Hz).

In the last decade, injectable dermal fillers are used also to shape, sculpt, and modify face features. The ideal next generation of dermal filler should provide the physician the possibility to sculpt and mold the patient face while giving lifting and void filling effect. Together with this, it should stimulate cell adhesion and proliferation (mainly dermal fibroblasts) promoting the formation of new extracellular matrix (ECM), including collagen deposition, that will eventually replace the injected material, leaving the patient with a long-lasting rejuvenated, young, and natural look.

With the development of recombinant human type I collagen, which exhibits the functional and biological properties of the native collagen molecule as produced in humans, and presents significantly improved rheological properties compared to tissue extracted collagen, aesthetic medicine is an obvious application. The photocurable dermal filler is a new generation of dermal filler that

gives the physician the ability to sculpt the target location and fix a desired shape of the injected composition by transdermal illumination and curing in place. Visible light is used to ensure safe penetration of light through the skin. The activation process leads to the formation of a mesh of covalently cross-linked rhCollagen entangled with cross-linked HA. The rhCollagen component is intended to provide biological benefit by promoting tissue regeneration and rejuvenation. The combination of photocurable rhCollagen (rhCollagen-MA) and cross-linked HA is designed for easy injection even in deep areas. Following the injection, once the desired contour shape is reached, light is shined transdermally on the treated area to cure the injected material and fix the final contour, as schematically represented in Figure 1 and elaborated in the video. [See Video (online), which describes the Photocurable Dermal Filler treatment concept (used with permission by CollPlant Ltd.).]

Although having relatively low stiffness (as reflected by G' value) during injection, G' of the injected material significantly increases following transdermal illumination and curing process, providing improved lifting effect. The final G' is determined by the modified collagen (rhCollagen-MA) concentration which is tailored according to the intended use (Fig. 2).

In addition, the curing process promotes adhesion of the gel to the surrounding tissue, avoiding events as filler migration. The filler will dynamically move together with the tissue allowing natural facial movements and expressions.

The ultimate requirement of a rejuvenation treatment is to induce skin regeneration, process where new autologous tissue eventually fills the void created by the aging process. In an animal study, it was demonstrated that a prototype photocurable formulation containing rhCollagen and cross-linked HA significantly enhances cell

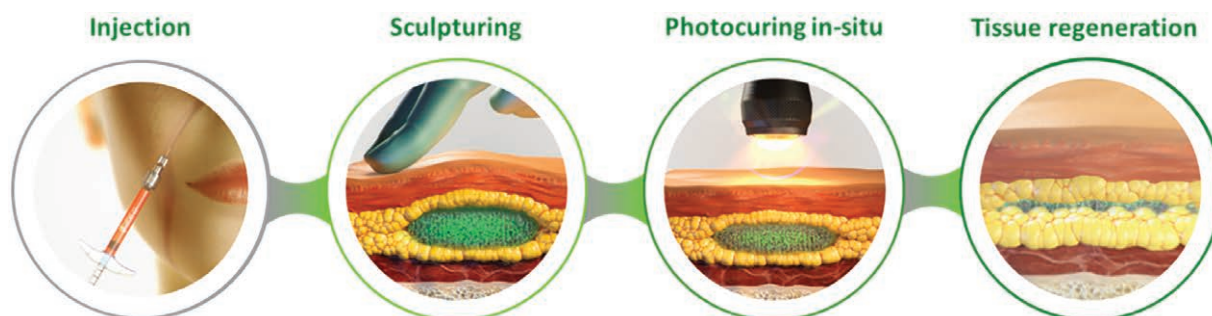


Fig. 1. Schematic representation of the injection, sculpting, photocuring, and tissue regeneration phases in a photocurable dermal filler application. Used with permission by CollPlant Ltd.

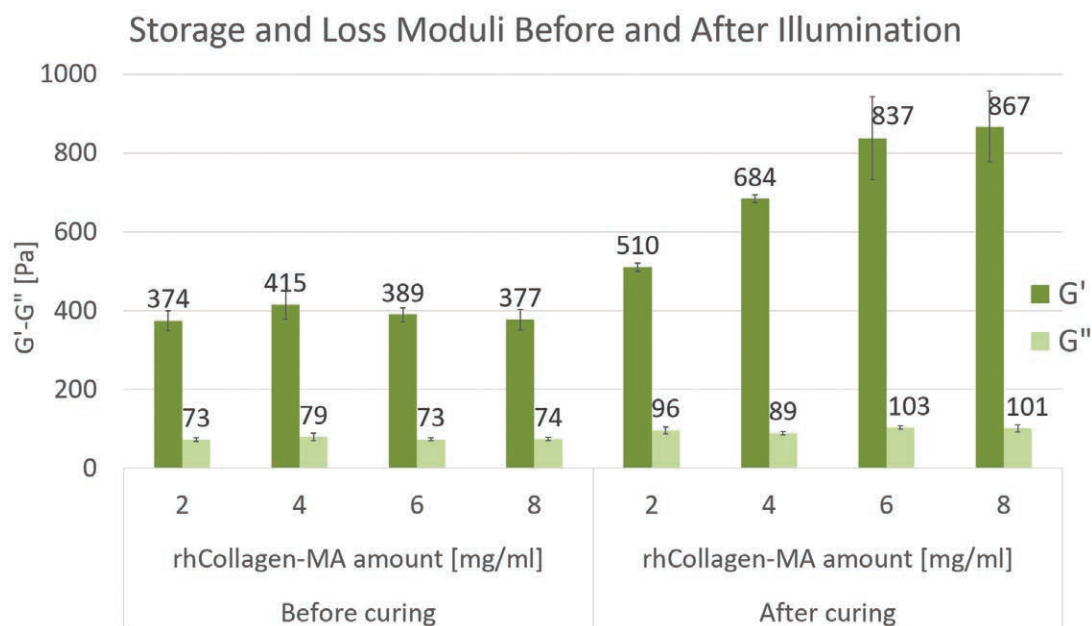


Fig. 2. Rheological measurements of photocurable dermal filler before and after the curing process: frequency sweep (0.05–100 Hz), controlled deformation (Deformation 0.8%) using plate-plate serrated geometry. In the graph: G' (storage modulus) and G'' (loss modulus) values before and after illumination (curing process) at frequency $f = 1$ Hz. Samples: 20 mg/ml cross-linked HA with 10 mg/ml rhCollagen (rhCollagen+rhCollagen-MA) with photoinitiator. The rhCollagen is a combination of different ratios of rhCollagen and rhCollagen-MA (8:2, 6:4, 4:6, 2:8). Following illumination G' increases with the increasing of rhCollagen-MA concentration.

proliferation (dermal fibroblasts) and new tissue regeneration within the injected material when compared to a standard of care HA-based filler (Figs. 3 and 4).

In light of the above, the photocurable dermal filler combining cross-linked HA and rhCollagen-MA opens a new paradigm in aesthetic medicine

where the physician can sculpt and precisely define the body contour, whereas the material fosters the body to restore its native tissue.

3D-PRINTED BREAST IMPLANT

Mammaplasty includes augmentation for increasing size, form, and feel of breast, and

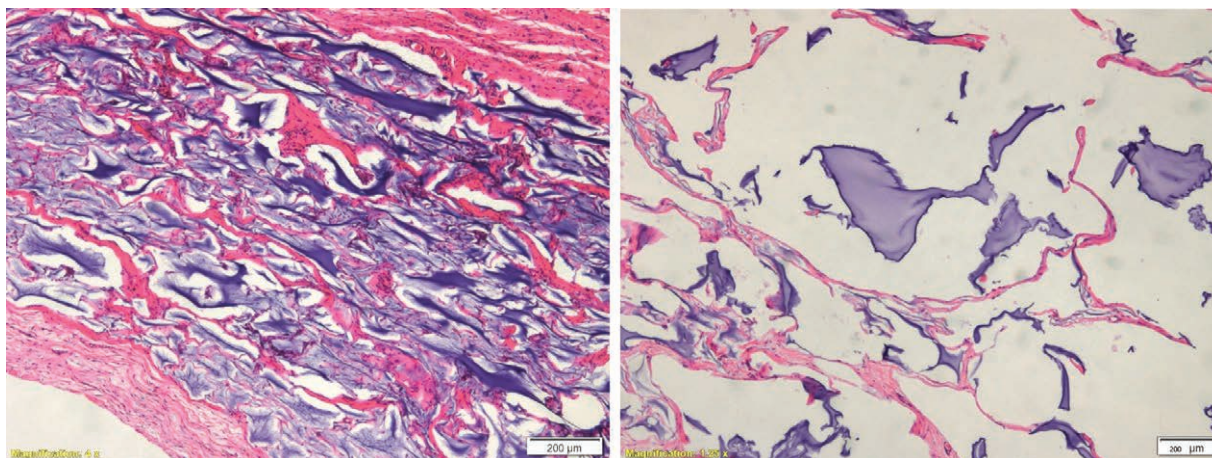


Fig. 3. Typical hematoxylin and eosin histology images of dermal filler in a rat model, 6 weeks after subcutaneous injection. (Left) Photocurable dermal filler composition containing 20 mg/ml cross-linked HA and 6 mg/ml rhCollagen-MA with photoinitiator followed by transdermal curing with white light; (right) standard of care (SOC) 20 mg/ml cross-linked HA filler. The photocurable dermal filler (left) significantly enhances cell proliferation and tissue regeneration, whereas almost no cells and no new tissue growth is observed in the SOC (right).

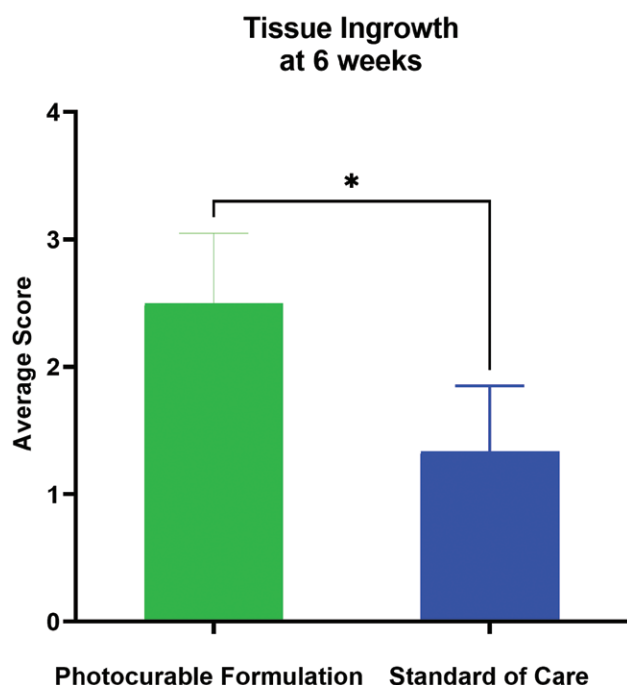


Fig. 4. Tissue ingrowth score (amount of new tissue grown inside the injected material) 6 weeks after injection. Photocurable dermal filler: 20 mg/ml cross-linked HA with 6 mg/ml rhCollagen-MA with photoinitiator followed by transdermal curing with white light. Standard of Care: 20 mg/ml cross-linked HA * $p < 0.05$, t test nonparametric.

reconstruction following damage to breast tissues by trauma, disease (breast cancer), and anatomic deformations. Traditional breast augmentation and reconstruction surgeries include prosthetic implants made of artificial fillers, that is, silicone gel, or other volume filling techniques including the use of tissue flaps, membranes, and fat grafting (alone or in combination). Many safety concerns are although associated with commercial breast implants as leakage of silicone filler leading to granulomas (inflammatory nodules), axillary lymphadenopathy (enlarged lymph glands in the armpit area)^{29–31} and T-cell lymphoma³² (breast implant-associated anaplastic large cell lymphoma, BIA-ALCL). Consequently, in 2016, the World Health Organization issued a specific warning for using silicone implants leading the number of procedures of breast augmentation, which has tripled since 1997, to gradually decline. According to the American Society of Plastic Surgeons in 2019, there was in the United States an 8% decrease in number of women undergoing breast augmentation²⁹ and a 15% increase in breast implant removals compared to 2018. In view of the current and growing demand for

breast implants, there is an unmet need for safe solutions.

CollPlant Ltd., a regenerative and aesthetic medicine company located in Israel, is currently developing a 3D-bioprinted implant for augmentation and reconstruction of breast tissue as an efficient alternative to traditional breast augmentation and reconstruction techniques. The 3D-bioprinted breast implant is a uniquely designed scaffold printed with a photocurable rhCollagen-based biological ink. The bioink formulation provides tissue regeneration and promotes neovascularization thanks to the rhCollagen, whereas biocompatible synthetic polymers provide the mechanical strength to withstand physiological and external loads. The implant design and bioink formulation are tailored to get specific physical and mechanical properties to resemble the natural breast tissue. To promote specific adipose tissue ingrowth inside the implant, the porous 3D scaffold is loaded with an injectable formulation composed of rhCollagen, to enhance cells infiltration and neovascularization, and biological additives specifically selected to promote adipogenicity. The 3D scaffold and filler will gradually degrade and be replaced by newly grown natural vascularized breast tissue free of any foreign material. An image of a small scale prototype of a 3D-printed scaffold designed for in vivo animal studies is provided in Figure 5. The porosity of the scaffold is controlled to get proper internal diffusion while the opening on the top is designed to receive the injectable formulation that enriches the scaffold with biological essential additives.

CONCLUSIONS

Type 1 collagen plays a major role in ECM functionality providing mechanical support and controlling biological signaling essential for tissue regeneration and homeostasis maintenance. Tissue quality is a central concept in aesthetic medicine: the newly grown or reconstructed tissue, being it skin, breast or any other, must reproduce the mechanical property of the healthy, juvenile original one. Cells require very specific conditions to synthesize the optimal ECM components that will constitute the new tissue. Regenerative Medicine can be defined as the science that aims to mimic nature, providing cells with growing conditions as similar as possible to the original ones. rhCollagen scaffolds surround cells with the same main ECM building block protein as the host organ promoting and enhancing cell functionality. The photocurable rhCollagen can form 3D structures with tunable mechanical properties

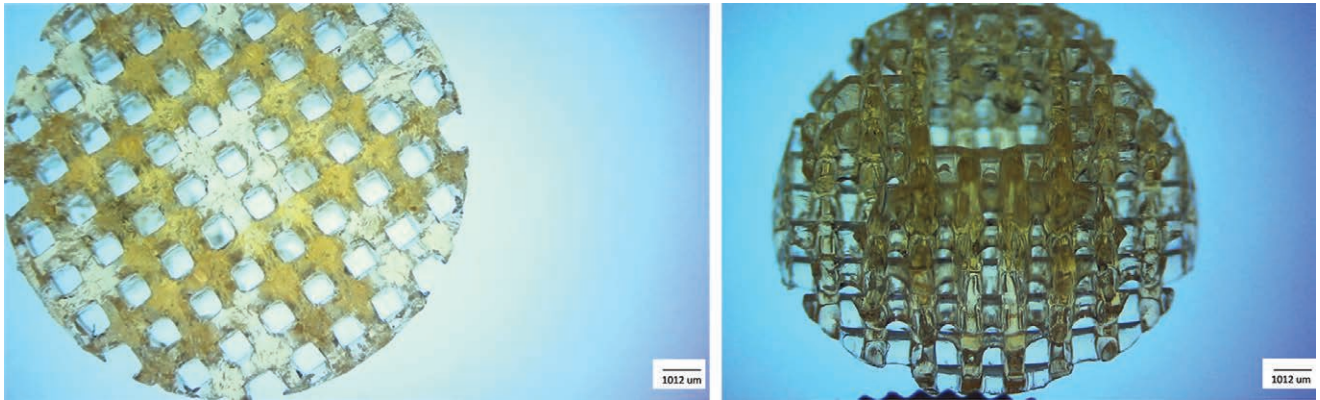


Fig. 5. Miniature breast implant prototype printed with DLP printer (LabFab DLP 3D printer, 3D Systems). The BioInk is a composition of synthetic polymers and modified rhCollagen.

according to curing conditions and formulation providing mechanical support in addition to the biological functions. In this article, we presented potential applications of the photocurable rhCollagen in two main aesthetic medicine treatments, facial lifting and sculpting, and breast augmentation/reconstruction. Although very differently, proper shape and tissue formation are essential in both treatments to achieve best results. The photocurable dermal filler introduces a new concept in contour sculpting using light as a trigger for in situ shaping. It gives the physician the possibility to sculpt and define the final shape via simple transdermal illumination of the injected material, fixing the desired contour. The illumination device under development will be designed to match different skin colors. The photocurable dermal filler allows smooth injection of a semiliquid filler with relative low stiffness. When illuminated with visible light, rhCollagen cross-links enhancing collagen stability and filler mechanical properties and resistance to facial deformation. At the same time, the rhCollagen promotes cells infiltration and neovascularization leading to new ECM and collagen deposition and long-lasting rejuvenation appearance. In the breast implant, the desired design is obtained via accurate 3D-printed scaffold thanks to a photocurable rhCollagen-based bioink formulation specifically developed to have unique rheological and biological properties. The 3D-printed breast implant is formulated with biological additives to promote the growth of new autologous adipose tissue. Many questions are still to be assessed as the longevity of the newly formed tissue and the development of appropriate surgery techniques; however, the concept of replacing silicon foreign implants with a collagen-based scaffold that will be populated by the patient cells,

and eventually substituted with ECM generated by those cells, eliminating the safety risks associated with current implants, is definitely a game changer in the breast augmentation/reconstruction context.

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DISCLAIMER

The photocurable dermal filler and the 3D-bioprinted breast implants are under development and are not commercially available.

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