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ORIGINAL ARTICLE

Combining topical dermal infused exosomes with injected calcium hydroxylapatite for enhanced tissue biostimulation

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Abstract

Background: Exosome research continues to flourish. Subsequent knowledge surrounding indications, dose–response, safety, efficacy, and the ability to combine exosome treatment as a "skin primer"—for biostimulation modalities such as calcium hydroxylapatite (CaHA), platelet-rich plasma (PRP), and platelet-rich fibrin matrix (PRFM) is growing rapidly. The objective of this study was to develop safe, reproducible methods of improving topical exosome absorption to enhance the quality of skin either by themselves, or in combination with injectable CaHA.

Methods: Under IRB Approval (International Cell Surgical Society: ICSS-2022-007), 40 patients were enrolled in this study. Twenty patients underwent facial biostimulatory dermal infusion alone, to determine if this method allowed adequate exosome absorption. Five patients underwent facial biostimulatory infusion followed immediately by Dilute CaHA injection (1:1 dilution) to the face. Five patients underwent exosome biostimulatory dermal infusion followed immediately by hyperdilute CaHA (dilution 1:4) injection to the neck. Five patients underwent Facial Dilute CaHA injection (1:1 dilution) alone, without dermal infusion. Five patients underwent neck hyperdilute CaHA injection (1:4 dilution) alone, without dermal infusion. All patients had pretreatment Quantificare 3-D photo-documentation and skin analysis (Quantificare, France). In all patients, the skin was first cleansed with a gentle glycolic acid facial wash (Gregory MD). To induce a "homing inflammatory environment" for the exosomes, sea salt exfoliation was performed (SaltFacial®, SaltMed, Cardiff, CA). A nitric oxide—generating serum (N101 Pneuma Nitric Oxide, Austin, TX) was then applied to act as an enhanced vehicle for absorption. A 3 MHz ultrasound (SaltFacial®, SaltMed, Cardiff, CA) was then utilized to further deepen the absorption of the nitric oxide serum. A topical emulsion containing equal volumes (1.0 cc containing 1 million) of exosomes (Kimera Labs, Miramar, FL), 25 units of botulinum toxin (Xeomin, Merz Aesthetics, Raleigh, NC) and hyaluronic acid (Belatero, Merz Aesthetics, Raleigh, NC) was mixed via back-and-forth propulsion in a 3-cc syringe. When adequately mixed, the emulsion was then applied to the treatment areas. The cavitating ultrasound was then used to aid in the absorption of the emulsion. The patients were then treated

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with high-intensity LED therapy (SaltFacial®, SaltMed, Cardiff, CA), utilizing the collagen restoration preset program of combination red (660 nm) near-infrared (930 nm) wavelength for 20 min. Post-treatment Quantificare analysis was performed at 15 and 30 days after treatment.

Results: Without exception, all dermal infusion alone and CaHA injection alone patients showed an improvement in the tone, quality, and texture of their skin. Quantificare results showed consistent improvement in wrinkles, pores, skin evenness, improved vascularity, and a reduction in oiliness and unwanted pigment. When employed as a skin primer prior to injections (CaHA), enhanced and more rapid results were seen.

Conclusions: Biostimulatory dermal infusion can be achieved utilizing topical placental mesenchymal stem cell-derived exosomes. These exosomes can be used alone, or mixed with ancillary ingredients such as botulinum toxin, hyaluronic acid dermal filler, and CaHA to customize and personalize treatments based upon individual patient needs. Topical absorption is enhanced with sea salt exfoliation, a topical nitric oxide-generating serum, and 3 MHz cavitating ultrasound. Post-absorption activity is enhanced with high-intensity LED treatment. The addition of CaHA injections after the topical exosome "priming of the skin" yielded enhanced skin quality faster than exosomes or CaHA alone.

KEYWORDS
biostimulatory, calcium, exosomes, hydroxylapatite, infusion

1 | INTRODUCTION

The fields of cellular medicine, regenerative and stem cell therapies continue to grow exponentially, particularly as it relates to therapeutics and diagnostics. Exosome research has progressed over the past two decades, culminating in thousands of peer-reviewed publications.[1] The focus of early research was primarily on functional disorders, particularly degenerative and autoimmune diseases.[2] In recent years, more research has emerged focusing on aesthetic and reconstructive surgical applications.[3,4] Many authors are focusing on the utilization of exosomes for treating aging skin, acne, alopecia, wound healing, and scar therapy, particularly hypertrophic and keloid scars.[5,6]

The mechanism of action of mesenchymal stem cells (MSCs) in regeneration remains under study.[7] Early hypotheses speculated engraftment and trans-differentiation with creation of new cells to effect repair. In 2006, Caplan described MSCs as "medicinal signaling cells" capable of releasing paracrine effectors which influence the body via immunomodulatory and trophic mechanisms.[8] These bioactive messengers can upregulate resident stem cells, which reside throughout all tissues of the body, and affect the phenotypic and physiologic expression of the immune system. MSCs are in constant communication through biologic signaling with surrounding cells and contain a feedback loop involving their own cell membranes. This complex signaling system allows stem cells to induce healing of damaged target cells within proximity without engraftment. This cellular communication is affected by messengers termed exosomes and microvesicles, collectively known as extracellular vesicles (EVs), or secratomes.

Exosomes measure 50–150 nm and are lipid membrane packets formed by a two-step budding process. First formed by inward budding of membranous vesicles in a multivesicular body, they fuse with the plasma membrane to release exosomes. Microvesicles are larger packets formed by direct budding of the plasma membrane. Both contain transmembrane proteins from their parent cells which aid in regulating uptake by other cells.[9] Exosomes contain messenger RNA (mRNA), microRNA (miRNA), and a multitude of proteins consisting of growth factors (Table 1) and immune factors (Table 2). When properly stimulated, they illicit homing, regenerative, anti-inflammatory, immunomodulatory, and anti-prostaglandin mechanisms.

Extracellular vesicles travel systemically without the risk of clumping. They do not demonstrate a first-pass effect into the lungs when administered intravenously as is commonly seen with MSCs. EVs can cross the blood–brain barrier without using mannitol.[9] Exosomes can evade the immune response as they contain no DNA, yielding no risk for malignant transformation or hypersensitivity reactions.[10]

An increasing number of younger and older patients are presenting for consultation in aesthetic offices. Within this patient population exists a desire for minimally invasive, non-surgical procedures seeking preventative aesthetic solutions to prolong the necessity for future corrective aesthetic therapies. This trend has fueled the current explosion in the expanding knowledge and study of placental mesenchymal stem cell-derived vesicles or exosomes.[1]

TABLE 1 Key immune and growth factors present in MSC exosomes.

BMP5	Stimulates Bone Growth
GDF15	Regulates inflammation, apoptosis, cell repair, and growth
OPG	Stimulates Bone Growth/Blocks Osteoclast Precursor Formation
G-CSF	Stimulates Bone Marrow to Procedure Granulocytes and Stem Cells
SCF	Responsible for Stem Cell and Melanocyte Growth
TGFß3	Most Important Anti-Inflammatory Protein. Converts Inflammatory T Cells into Anti-Inflammatory Regulatory T Cells.
VEGF	Stimulates Formation of Blood Vessels
ICAM-1	Binds Inflammatory Ligands on White Cells
IL-1RA	Binds and Sequesters the Inflammatory Cytokine IL-1
IL-6	Responsible for Macrophage Activation
IL-10	Anti-Inflammatory Cytokine responsible for Immunomodulation and Regulatory T Cell Conversion
MCP-1	Recruits Mononuclear Cells to Treatment Area
MIP-1	Also known as CC1-4, Recruits Mononuclear Cells to the Treatment Area
PDGF-BB	Growth Factor Used to Stimulate Healing in Soft and Hard Tissues
TIMP1 and TIMP2	Blocks Cartilage and Extracellular Matrix Degradation, Important for Cartilage Repair
HGF	Involved in Organ Regeneration and Wound Healing
GDNF	Promotes Survival of Neurons
BDNF	Supports Survival of Neurons and Encourage Growth
FGF	Potent Growth Factors Affecting Many Cells
TNFR1	Binds and Inactivates the Inflammatory cytokine TNF-*a*

TABLE 2 Key mRNA present in MSC exosomes.

IL-1RA
TIMP1 and TIMP2
TNFR1 and TNFR2
Numerous Histone Deacetylase mRNAs
GDF11—Potent anti-aging agent
GDF15—Regulates inflammation
IGFBP2One of six IGF binding proteins that bind IGF-1 and IGF-2
IGFBP3
IGFBP4—Reportedly anti-tumorigenic effects against prostate cancer, colon cancer, and glioblastoma
IGFBP6
OPG
SCFR
TGF-ß1 and TGF-ß3 VEGF
VEGFR-2
BMP4—Involved in bone and cartilage development, fracture repair, and muscle development
BMP7—Important in bone homeostasis PTEN - A potent tumor suppressor gene Numerous Key miRNA

Mesenchymal Stem Cell Exosomes (MSC exosomes) are produced by stem cells of connective tissue lineage which is the origin of skin, hair, bone, muscle, and cartilage. MSC exosomes are different from adult bone marrow exosomes, which has a preponderance of hematopoietic stem cell exosomes. Another source of exosomes is derived from amniotic fluid. These are composed of primarily maternal epithelial cell exosomes. It is hypothesized that the ability of MSC exosomes to induce the synthesis of connective tissue is the basis for remarkable clinical benefits resulting from stem cell therapy.[8]

The advantage of perinatal MSC exosomes over exosomes from aged autologous or allogenic progenitor cells resides in the fact that with age, the number, and function of MSCs in our tissue declines. Aged autologous progenitor cells also produce less than 40% of the cytokines and differing miRNAs than perinatal MSCs. This secretome advantage of younger exosomes is therefore significant.

Internationally, early IRB-approved research examined various delivery options of exosomes. It was found that if using a sterilized purified exosome product, safe delivery was possible topically, aerosolized inhalation, injection intradermally, subcutaneously, intravenously, and intra-articular.[5] At the time of the writing of this paper, no North American Company producing exosomes or exosome-derived growth factor products possesses FDA approval for any indication. This led the author to seek to maximize the topical absorption of exosomes for the purposes of improving the appearance of skin.

The concept of tissue regeneration has come to the forefront of many investigations. With proper homing targets in place, exosomes possess anti-inflammatory, regenerative, immunomodulatory, and anti-prostaglandin effects unto themselves.[1] When applied topically, MSC exosomes have been shown to improve the quality, tone, and texture of skin by reducing wrinkles, pores, oiliness, unwanted pigmentation, and improving skin evenness, and vascularity.[5] Dermal remodeling with Type 1 neocollagenesis and elastin production has been observed.[5]

The volumization benefits and mechanisms of calcium hydroxyapatite (CaHA, Radiesse; Merz Pharmaceuticals GmbH, Frankfurt, Germany) have been well described.[11–13] Subsequent reports reported noticeable improvements in skin firmness and appearance

after the injection of diluted CaHA in the arms, abdomen and thighs.[14] Further work demonstrated this product to possess the ability to provide immediate volume correction followed by regeneration of new tissue formation through neocollagenesis, elastin production, angiogenesis, and dermal cell proliferation.[15–17] It was found for subdermal or supraperiosteal placement.

Subsequent off-label techniques used diluted or hyperdiluted CaHA to stimulate dermal regeneration without creating a volumizing effect.[18,19] The subsequent enhanced firmness and turgor led to a smoother and brighter appearance to the skin.

Given the biostimulation capabilities of topical exosomes and injected CaHA, the author wanted to examine the synergistic potential of the two modalities when performed concurrently. This led to studies seeking the maximum absorption efficiency of topically applied exosomes, and ways to enhance their intradermal activity. This equated to the removal of the stratum corneum barrier, followed by rapid optimal absorption and activation.

A proven enhanced vehicle for absorption is a topical nitric oxide-generating serum. Nitric oxide (NO), is an important biological messenger in human physiology. This multifactorial signaling molecule takes part in the control of vasodilation[20] and is involved in the inhibition of platelet aggregation and platelet adhesion to vascular endothelium.[21,22] Several human skin studies have shown that NO is involved in the proliferation and differentiation of epidermal cells, regulation of innate immune reactions and inflammatory responses, the control of allergic skin manifestations, microbicidal activity, and antigen presentation.[23,24] NO is a key molecule in regulating wound healing and tissue regeneration due to its gene regulatory properties, as well as its influence on the proliferation and differentiation of fibroblasts, keratinocytes, monocytes, and macrophages.[25–31]

Bryan has written extensively on the science of NO and its importance in human health.[32–34] NO is a short-lived free radical produced enzymatically by nitric oxide synthase endogenously. By 40 years of age, endogenous production is decreased by 50% in most adults. Given the importance of NO, and its natural depletion over time, a patented NO lozenge, Neo40 (human, Austin, TX), was formulated. Studies showed that the NO lozenge could modify cardiac risk factors in patients over 40, reduce triglycerides, and reduce blood pressure. The importance of nitric oxide in cellular medicine, regenerative, and stem cell therapy has also been highlighted in the literature.[4] The role of NO in stem cell biology has revealed novel strategies for optimizing metabolism for regenerative and healing outcomes.

The success of oral NO repletion led to studies looking at topically applied NO generating formulations.[35] (N1o1, Pneuma Nitric Oxide, Austin, TX) It has a stoke radius of 3-4A that can diffuse across most biological membranes. The diffusion coefficient of NO has been shown to be comparable to other small diatomic molecules such as dioxygen and carbon monoxide. This is sufficient for NO to penetrate the dermis and reach the microcirculation of the upper horizontal plexus and the upper dermis after topical application.[36] The transcutaneous application in vivo of a formula generating NO was shown to significantly increase circulatory hemodynamic parameters.[37] Subsequent work in the Aesthetic and Reconstructive patient population showed the efficacy of NO in improving skin appearance,[38] reducing the effects of acne,[39] accelerating wound healing,[40] and aiding scar therapy.[38] These studies also showed its ability to act as an enhanced vehicle of absorption if applied prior to other products.

To further optimize the skin for topical absorption of exosomes, exfoliation of the stratum corneum and the creation of an inflammatory milieu can be achieved with microdermabrasion. Studies have shown that exfoliation with sea salt has benefits over traditional aluminum hydroxide crystals.[41] Topical sea salt is antibacterial, antiviral, antifungal, and immunomodulatory, drawing out skin impurities. It is hydrophilic, aiding in protection of the hydration barrier.

Cavitating ultrasound and dermal ionophoresis have shown scientific benefits to skin health.[42] Traditional topical delivery of products to tissue is limited by epidermal permeability dependent upon the molecular weight of the product applied, and the existing circulation of the recipient tissue bed. Cutaneous ultrasound has been shown to improve circulation and increase tissue permeability, thereby increasing depth of penetration and absorption of topically applied substances.[43] When focused deeper, with higher intensity, cavitating ultrasound can reduce adipocytes and improve lymphatic drainage.[44]

Once delivered into the dermis, enhancing exosome activation and subsequent tissue remodeling can be achieved with LED treatment. LED therapy has been investigated for skin health.[45] The most commonly used and beneficial wavelengths are blue (414 nm), which has been shown to be bactericidal, red (660 nm), which can reduce inflammation and induce cellular proliferation, amber (590 nm) which has anti-inflammatory benefits, and infrared (830 nm), which improves vascularity and collagen production.[45]

While studies have examined exosomes, CaHA volumization, CaHA biostimulation, nitric oxide, sea salt, cavitating ultrasound, ionophoresis, and LED therapy, this is the first study to examine a multimodality approach employing the techniques in a synergistic manner to examine exosome biostimulation alone, and whether together with CaHA, an accelerated and qualitatively improved result can be achieved.

2 | METHODS

Forty patients were enrolled in this Study. There were 35 females and 5 males. Ages ranged from 34 years to 72 years. Twenty patients underwent exosome biostimulatory infusion alone. Five patients underwent facial exosome biostimulatory infusion followed immediately by dilute CaHA (dilution 1:1) injection to the face. Five patients underwent exosome biostimulatory dermal infusion followed immediately by hyperdilute CaHA (dilution 1:4) injection to the neck. Five patients underwent injection of dilute CaHA (dilution 1:1) to the face without exosomes. Five patients underwent injection of hyperdilute

CaHA (dilution 1:4) to the neck without exosomes. Facial CaHA injections were performed with the needle technique. Neck CaHA injection were performed with the cannula technique.

In all groups, pretreatment Quantificare 3-D photodocumentation and skin analysis (Quantificare, France) was performed (Figure 1). The skin was first cleansed with a gentle glycolic acid facial wash (Gregory MD). To induce a "homing inflammatory environment" for the exosomes, sea salt exfoliation was first performed (SaltFacial, SaltMED, Cardiff, CA).

In all groups, a nitric oxide-generating serum (N101 Pneuma Nitric Oxide, Austin TX) was then applied to act as an enhanced vehicle for absorption. A 3 MHz ultrasound (SaltFacial SaltMED, Cardiff, CA) was then utilized to further deepen the absorption of the nitric oxide serum.

In all groups, a topical emulsion containing (1.0 cc, 1 million) of exosomes (Kimera Labs, Miramar, FL), 1.0 cc (25 units) of incobotulinumtoxinA (Xeomin, Merz Aesthetics, Raleigh, NC) and 1.0 cc of hyaluronic acid (Belotero Balance, Merz Aesthetics, Raleigh, NC), was mixed via back-and-forth propulsion in a 3-cc syringe. When adequately mixed, the emulsion was then applied to the treatment areas. The cavitating ultrasound was then used to aid in the absorption of the emulsion.

The patients were then treated with high-intensity LED therapy (SaltFacial, SaltMED, Cardiff, CA), utilizing the collagen restoration present program of combination Red (660 nm) Near Infrared (930 nm) wavelengths for 20 min. Post-treatment Quantificare analysis was performed 30 days after treatment.

The exosomes utilized (Kimera Labs, Miramar, FL) were a suspension of isolated and purified, c-section donated, placental, mesenchymal stem cell-derived extracellular vesicles suspended in a saline solution. The product is manufactured in two concentrations: standard and a 3X concentrate. Both products have been available for many years and have been proven to be safe, consistent, effective, and reproducible from lot to lot. To ensure the safety of the finished vial product (FVP), donor eligibility is first established through review of medical records, social behavior, physical examination, and serological blood tests to verify the absence of relevant communicable diseases. Table 3 lists the Donor Safety Testing procedures.

Once donor eligibility is deemed acceptable and released, the isolated Mesenchymal Stem Cells (MSC) are characterized to ensure identity and safety. Once the Master Cell Bank (MCB) has been fully characterized, and safety confirmed, then manufacturing of the FVP proceeds.

Both manufacturing and all analytical testing of these exosomes are performed under strict cGMP guidelines and regulations as per 21 CRF210, 211, as well as GLP HCT/P Regulations and guidance as per 21 CFR 1271. Table 3 lists donor safety testing/evaluation. Table 4 lists the testing performed on either the finished bulk product (FBP) or the FVP.

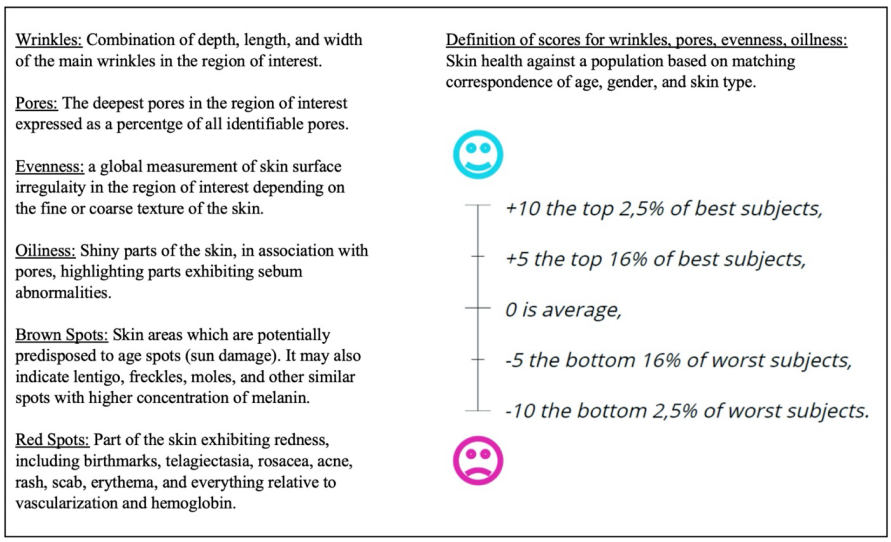


FIGURE 1 Quantificare skin care definitions and analysis.

TABLE 3 Donor's safety testing/evaluation.

CMV IgM (EIA) Biorad	Hepatitis B Surface Ab	Syphilis Screening Nontreponemal	WNV
CMV IgG (EIA) Biorad	Hepatitis C Virus Ab	Ultrio Elite HBV	ZIKA Virus
CMV Total Ab	HIV-1/HIV-2 Plus O	Ultrio Elite HCV	JCV PCR
Hepatitis B Core Total Ab	HTLV I/II AB	Ultrio Elite HIV-1/2	COVID-19

Quality Attribute	Test
Quality	• Appearance • Size • rtPCR
Strength	• Protein Concentration by A280nm • RNA Extraction
Purity	• SEC-HPLC • SDS-PAGE non-reduced Coomassie Blue Stained • RNA Extraction
Identity	• Western Blot for specific surface markers • ONI Imaging for specific surface markers • RRT by SEC-HPLC
Safety	• Mycoplasma • Bacterial Endotoxin • Sterility by Direct Inoculation • Patriciate Matter in Injections by Light Obstruction Method by USP <788> for Therapeutic Protein Injections
Biological Activity	• Cell-Based ELISA Potency Assay • rtPCR

TABLE 4 FBP and FVP quality attributes and testing.

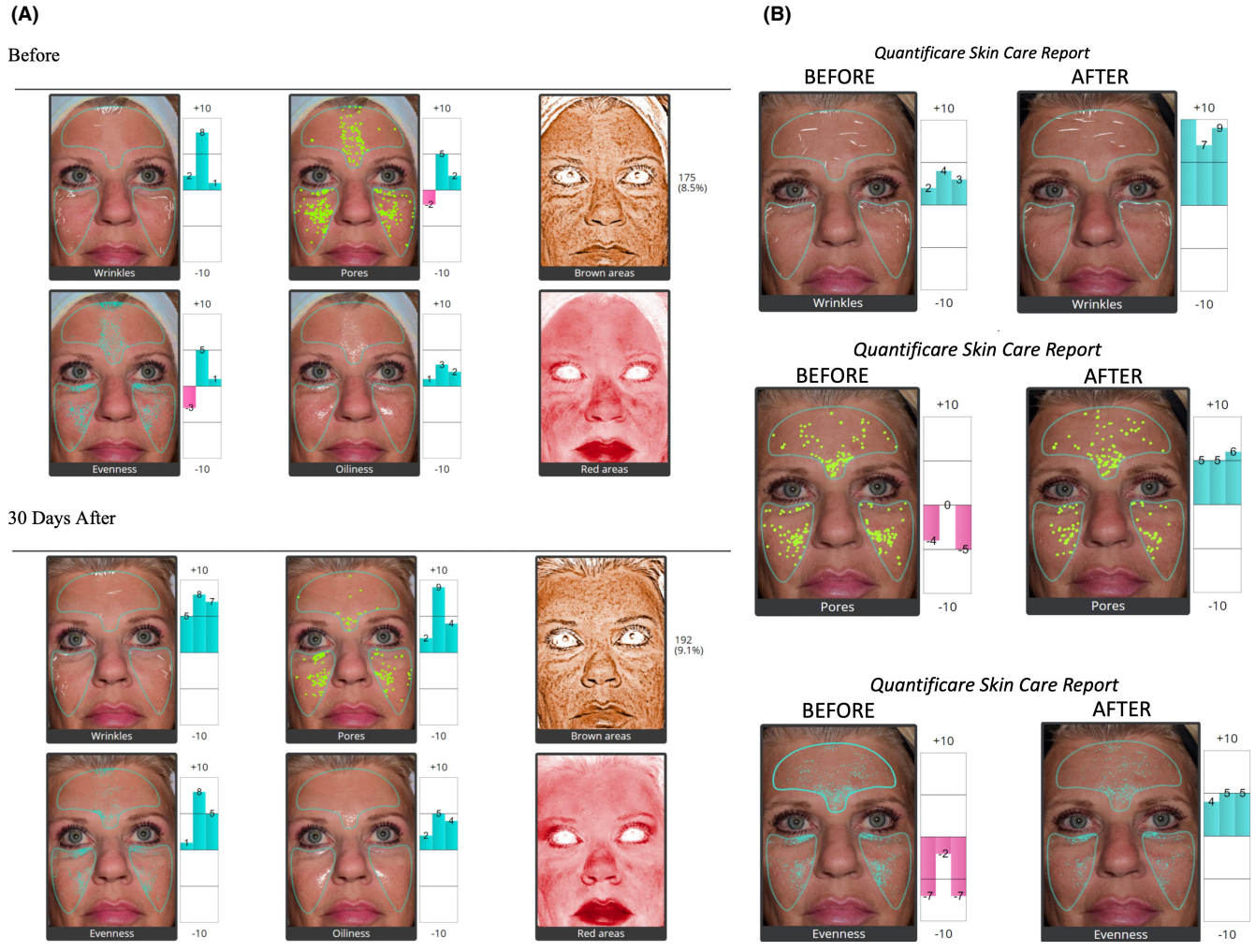


FIGURE 2 (A) Biostimulatory dermal infusion. (B) Biostimulatory dermal infusion (continued)—after at 30 days post-treatment. (C) Biostimulatory dermal infusion (continued)—after 30 days post-treatment.

3 | RESULTS

There were no allergic reactions, hypersensitivity reactions, or adverse events noted in any treatment group. No patients were lost to follow-up.

Linear Analog Results revealed 16 of 20 Dermal Infusion Patients to be very satisfied with the results with 4 of 20 reported being satisfied. There were no dissatisfied reports. Patients in the Infusion Group uniformly were pleased that there was no pain associated with the treatment.

Uniformly, all Dermal Infusion Patients and CaHA injection patients alone, showed an improvement in the tone, quality, and clarity of their skin with a reduction in fine lines, pores, pigment oiliness, and an improvement in texture and vascularity.

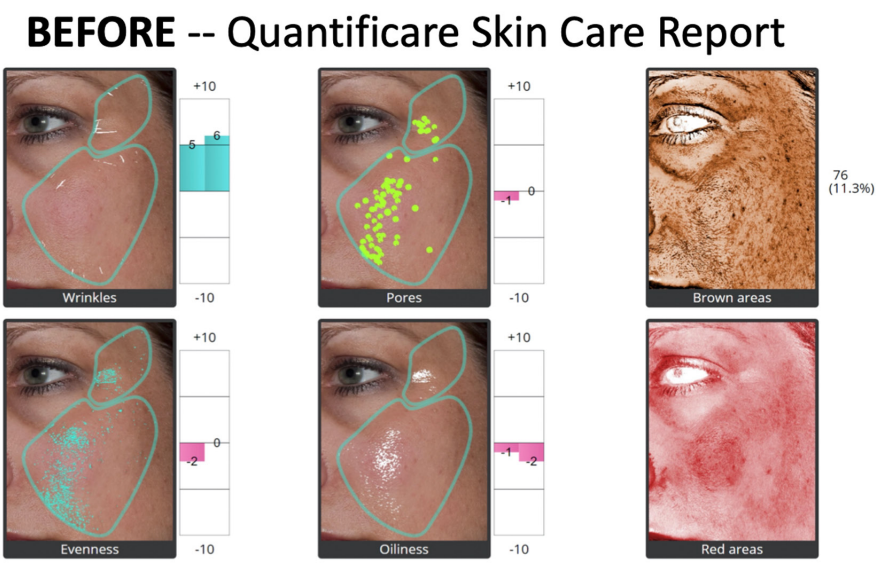
FIGURES 2–4 show representative patients' Quantificare results at 30 days.

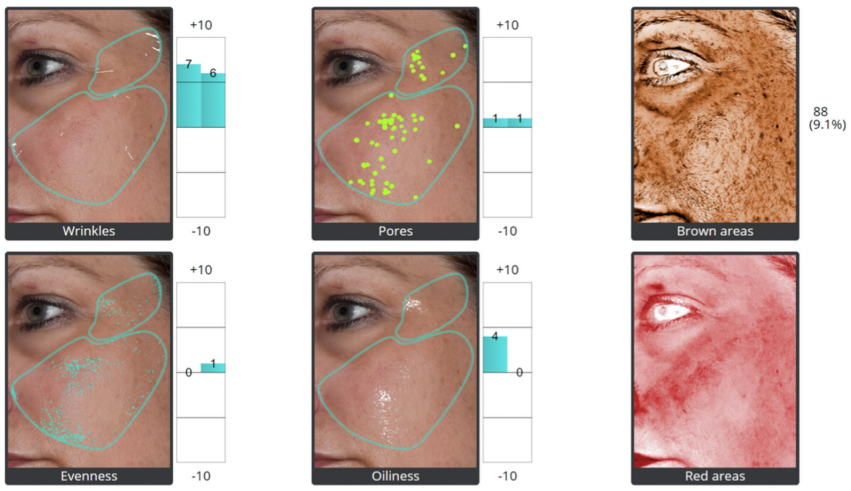
The patients who had dermal infusions immediately prior to CaHA injections displayed an earlier and more enhanced response (Figure 5) than the CaHA facial injections alone at 30 days (Figure 6).

4 | DISCUSSION

There are several important findings within this Study. As previously cited, the concept of "tissue regeneration" by diluting or hyperdiluting CaHA and injecting more superficial than for volumization has been demonstrated.[16] Histology has shown neocollagenesis and elastin production at 4 months.[16] Immunohistochemical analysis of biopsy tissue demonstrated significant increases in collagen 1 and collagen 3.[16] Staining for elastin and angiogenesis increased significantly at 4 months.[16] This equates with an increase in dermal thickness and would correspond to improvements in Quantificare Analysis scores.

FIGURE 3 Biostimulatory dermal infusion—before and 30 days after.





Studies have shown the anti-inflammatory, regenerative, immunomodulatory, and anti-prostaglandin effects of MSC exosomes, with proper homing, due to the multitude of robust growth factors, including TGFB3 and VEGF.[1] Histological verification of neocollagenesis, elastin production and angiogenesis has been shown in numerous exosome studies (4, 5, 6). These studies utilized methods

FIGURE 4 Biostimulatory dermal infusion—before and 30 days after.

such as microneedling, needle radiofrequency, and fractionated CO_2 laser to first create absorption channels prior to the topical application of the exosomes. Each of these modalities required topical anesthesia or regional blocks to allow the patient to be comfortable.

The ability to enhance dermal absorption of topically applied substances while maintaining their efficacy is significant. The avoidance of discomfort and pain in the clinical setting is priceless for many patients for whom pain tolerance is low. To our knowledge,

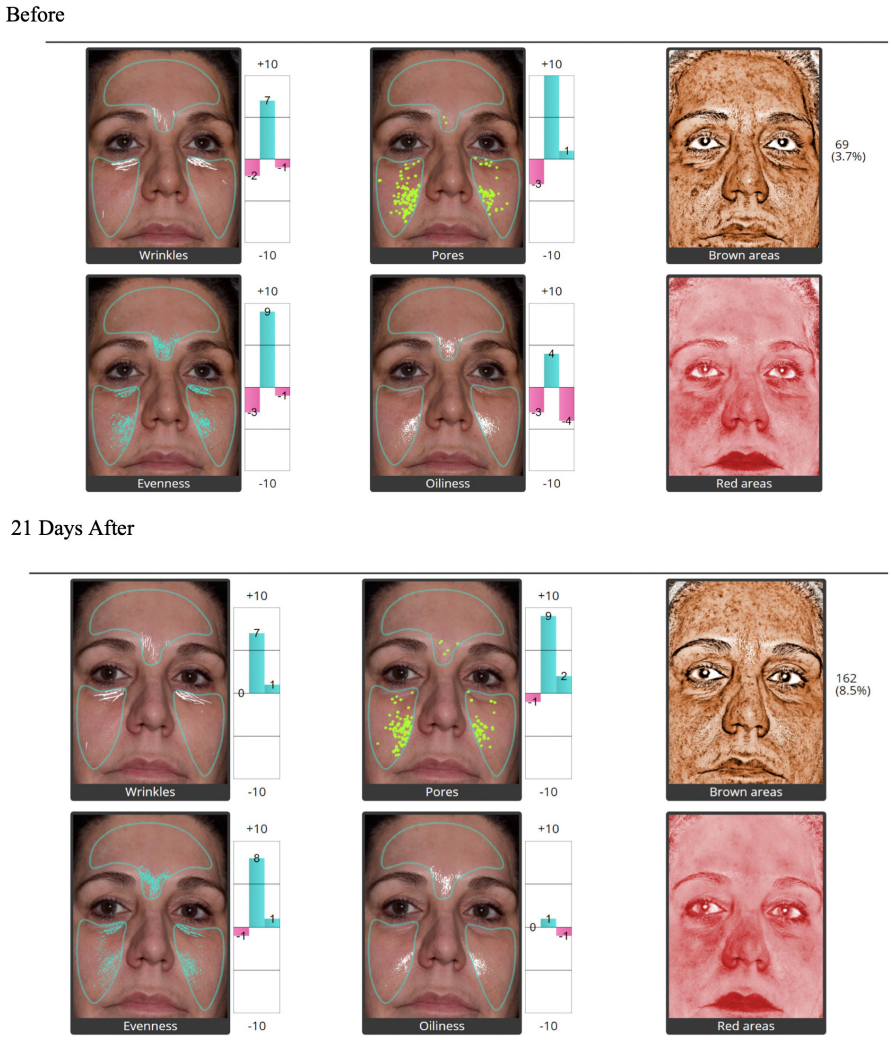


FIGURE 5 Biostimulatory dermal infusion + hyperdilute CaHA.

this is the first publication that shows preservation of the efficacy of exosomes after dermal infusion without breaking the surface of the skin in some manner.

Several factors play a synergistic role in achieving that success. Priming the skin by first removing the stratum corneum with sea salt exfoliation removes the outermost barrier to true dermal infusion. The next boost to absorption is the increased vascularity, through vasodilation and capillary recruitment afforded by the nitric oxide-generating serum. Additionally, nitric oxide boosts the biostimulatory efficacy of any applied substance due to its inherent ability to promote fibroblast, keratinocyte, monocyte, and macrophage proliferation and differentiation.[38] The antibacterial, antiviral, and antifungal properties of sea salt and nitric oxide give added protection to the biostimulatory diffusion process.

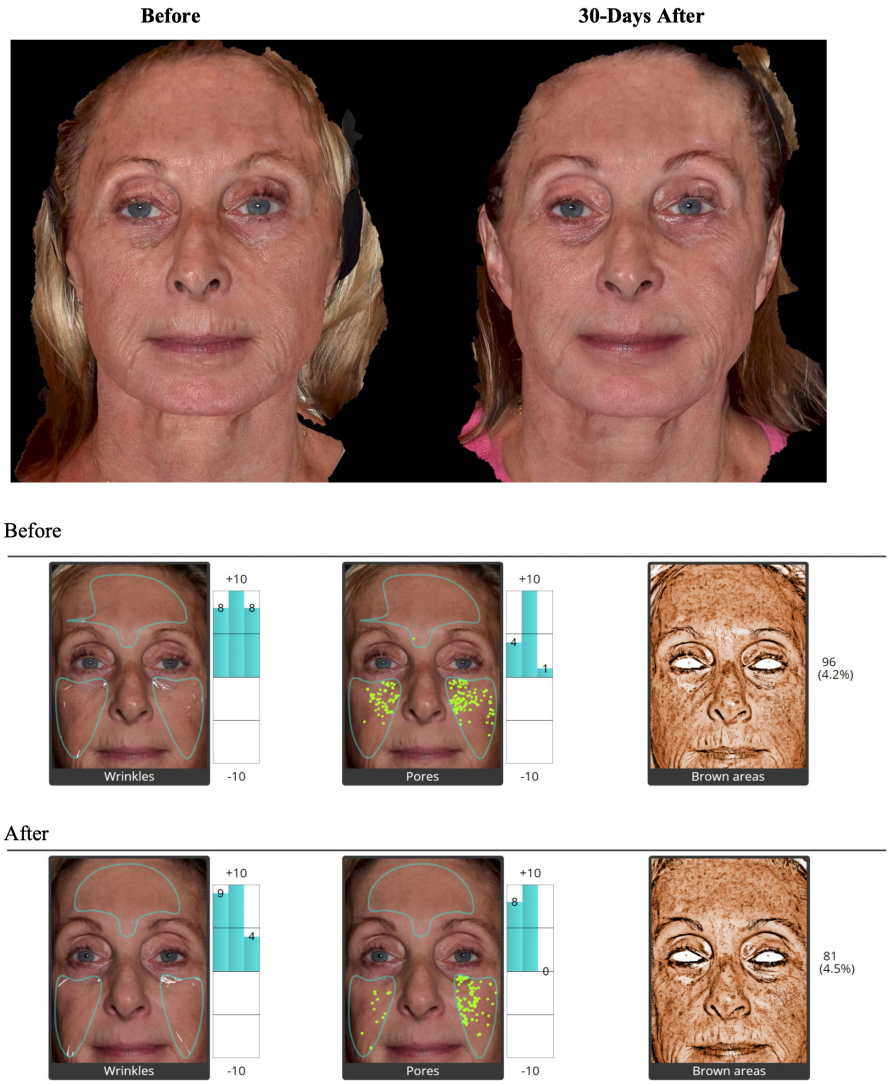
After the tissue is primed, the utilization of the 3 MHz ultrasound to propel the exosomes deeper into the dermis assures adequate depth of penetration for optimal stimulation by the growth factors. 3 MHz provides pulsed ultrasound delivery, yielding a cavitation effect, increasing intracellular spaces, thereby propelling agents through what remains of the stratum corneum, increasing passive epidermal and dermal permeability to topically applied agents.[41]

If ultrasound is focused on subcutaneous tissue, the tissue can reach temperatures exceeding 60°C, yielding small coagulation points to a 5-mm depth within the mid to deep reticular dermis, while leaving the epidermis and papillary dermis unaffected.[42] This acts as an additional exosome "booster", as this correlates with a "skin polishing" or an "air brushing" effect, yielding a further reduction in fine lines, increased quality and clarity of skin, and improved permeability with barrier restoration.[41]

Finally, once the "seeds have been planted" (exosomes), it makes sense to "fertilize the garden". The high-intensity LED combination treatment of Red (660 nm) which stimulates mitochondria and fibroblasts, as well as increasing cellular proliferation simultaneous with near infrared (930 nm) which causes vasodilation and fibroblastic proliferation facilitates exosome activity.[5]

Much work has been done by the author creating personalized (as every patient is different), customized (as every patient's problem is different) topical mixtures for the purposes of Biostimulatory Dermal Infusion. Ensuring ingredient stability and maximal dose–response curves which are safe and reproducible are the main challenges. Depending upon the problem (aging skin, acne, melasma, eczema, psoriasis), the ingredients differ and will be highlighted in future publications.

FIGURE 6 CaHA alone.



For aging skin, combining exosomes with highly hydrophilic molecules of hyaluronic acid rapidly accelerates a "plumping effect" yielding a more rapid visual response. The hyaluronic acid absorption coefficient is improved with the addition of gold nanoparticles added to the mixture. It is postulated that the hyaluronic acid also acts as a scaffold, like PRP, augmenting the exosome response. The addition of botulinum toxin into the epidermis and dermis relaxes the smooth muscle in the sweat glands, further softening fine lines.

An exciting finding in this study is the apparent improved biostimulatory effect of the dilute and hyperdilute CaHA by first "priming" the skin with the exosome/hyaluronic acid/botulinum toxin "soup." Consistent improvement of the Quantificare metrics by 30 days or sooner in the "priming" population suggests a piggyback effect via the combination approach. This warrants further study of this phenomenon.

5 | CONCLUSIONS

The utilization of MSC exosomes for the purposes of tissue regeneration via angiogenesis, neocollagenesis, and elastin production is possible via simple dermal infusion, without creating invasive absorption channels with instruments such as microneedling. Dermal infusion is optimized by employing a topical nitric oxide-generating serum prior to application as well as sea salt exfoliation, cavitating ultrasound for added propulsion, and LED treatment for exosome function optimization (SaltFacial, SaltMED, Cardiff, CA). By priming the epidermis and dermis with an exosome/hyaluronic acid/botulinum mixture prior to the injection of dilute or hyperdilute CaHA, an accelerated, augmented result is achieved.

The fields of cellular medicine, regenerative and stem cell treatments are growing exponentially and will continue to help the treating Physician take the best care of their patients.

CONFLICT OF INTEREST STATEMENT

No conflict of interest.

ETHICAL APPROVAL

This article is not published elsewhere. The author has no conflicts of interest. This Study was funded by the author. There are no co-authors. Conducted under IRB approval: (ICSS-2022-007), with all patients signing appropriate informed consents.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Chernoff Cosmetic Surgery at https://www.drchernoff.com, reference number 1120221552.

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