A novel professional-use synergistic peel technology to reduce visible hyperpigmentation on face: Clinical evidence and mechanistic understanding by computational biology and optical biopsy

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Abstract
Topicals and chemical peels are the standard of care for management of facial hyperpigmentation. However, traditional therapies have come under recent scrutiny, such as topical hydroquinone (HQ) has some regulatory restrictions, and high concentration trichloroacetic acid (TCA) peel pose a risk in patients with skin of colour. The objective of our research was to identify, investigate and elucidate the mechanism of action of a novel TCA- and HQ-free professional-use chemical peel to manage common types of facial hyperpigmentation. Using computational modelling and in vitro assays on tyrosinase, we identified proprietary multi-acid synergistic technology (MAST). After a single application on human skin explants, MAST peel was found to be more effective than a commercial HQ peel in inhibiting melanin (histochemical imaging and gene expression). All participants completed the case study (N = 9) without any adverse events. After administration of the MAST peel by a dermatologist, the scoring and VISIA photography reported improvements in hyperpigmentation, texture and erythema, which could be linked to underlying pathophysiological changes in skin after peeling, visualized by non-invasive optical biopsy of face. Using reflectance confocal microscopy (VivaScope®) and multiphoton tomography (MPTflex™), we observed reduction in melanin, increase in metabolic activity of keratinocytes, and no signs of inflammatory cells after peeling. Subsequent swabbing of the cheek skin found no microbiota dysbiosis resulting from the chemical peel. The strong efficacy with minimum downtime and no adverse events could be linked to the synergistic action of the ingredients in the novel HQ- and TCA-free professional peel technology.

KEYWORDS
acne, hydroquinone-free, hyperpigmentation, melasma, microbiome, molecular docking, multiphoton tomography, photodamage, reflectance confocal microscopy
1 | INTRODUCTION

Globally, there is a burden on dermatologists and skin care professionals to manage the influx of patients with hyperpigmentation (HP) disorders and skin lightening (SL) demand for cosmetic reasons. Topicals and chemical peels using hydroquinone (HQ) and trichloroacetic acid (TCA) are the dermatology standard first- and second-line therapy to manage HP and SL, respectively. They have proven efficacy in treatment of hyperpigmentation conditions such as melasma and post-inflammatory hyperpigmentation (PIH). HQ has more than 40 years of safety and efficacy history. Although no safety concern is reported on humans, prolonged use of HQ is associated with skin irritation, erythema and ochronosis or discoloration. Consequently there are research efforts, though limited, to find HQ-free alternative technologies to meet demands of people and professionals to manage HP and SL.

Chemical peeling is among the most popular non-surgical dermatology procedures. The use of Jessner’s solution for superficial peeling, and its combination with trichloroacetic acid (TCA) to enhance depth of penetration and efficacy has a long history of use by dermatologists and plastic surgeons. Jessner’s and TCA peels are often combined by dermatologists for a superficial TCA peel which is generally well tolerated and effective to treat melasma and PIH. However, TCA is a strong caustic and protein coagulation agent, hence, TCA peeling has major downtime (cosmetically non-visible reactions like frosting and exfoliation lasting 1 week or so), and less tolerated in particular by skin of colour. Although chemical peel is expected to work through controlled chemical injury action, unexpected severe adverse events like permanent injury and infections may occur from strong deep peels using phenol or high-strength TCA, and the risks are particularly high if the peeling is performed by non-physician ancillary staff. The micro lesions or wounds after chemical injury from peel may serve as a habitat for microflora. Depending on the degree of the chemical injury and the regrowth microflora, chemical injury may be followed by normal recovery to restoration or abnormal healing resulting in infection. To improve outcomes, dermatologists need to better understand histological and molecular changes after treatment; however, it is challenging in aesthetic medicine/procedures where taking excision of facial skin tissue (biopsies) is unnecessary.

Non-invasive optical biopsy and computational molecular modelling (predictive biology) can help us decipher the underlying anatomical and molecular changes in skin after treatment, without the need of invasive procedures. Using two or more therapeutics (combination/synergy therapy), rather than a single agent has proven to be an effective way to treat skin HP disorders. One successful example is Tri-Luma®, a combination of three drugs (HQ, tretinoin and fluocinolone acetonide), which has emerged as more effective treatment than HQ monotherapy for melasma and PIH. Combination treatment in chemical peeling is an effective and convenient method to manage HP, rather than a series of peelings over months using a single peeling agent.

The overarching goal of our well-structured holistic research was to use state-of-the-art dermatology research techniques to identify, investigate and clinically test a novel TCA- and HQ-free multi-acid synergistic technology (MAST) to manage all common types of facial hyperpigmentation in all Fitzpatrick skin types.

2 | METHODS

2.1 | In silico computational biology

Following the previous reports, molecular docking (MD) and molecular dynamic simulations (MDS) were done using open access software Autodock Vina 1.2.2 and GROMACS v.2019.2, respectively. A high performance computer was used to screen and understand molecular interactions of depigmentation and peeling agents with human tyrosinase enzyme (hTYR).

2.2 | In vitro enzyme assay

The activity of leads from in silico screening were tested on tyrosinase enzyme following manufacturer’s protocol (Sigma kit, catalogue#MAK257). Following our previous report, the synergy was investigated by testing mixtures of the leads at specific ratios and confirmed using open-access software CompuSyn from ComboSyn, Inc. USA. The synergy was defined as the cumulative effect of the mixture being more than the additive effect of the test agents when tested independently, and the CompuSyn value of combination index is <1.

2.3 | Ex vivo comparative study on skin explants

Human skin biopsies/explanted were washed, acclimatized and treated with the peel solution (commercial HQ-KA peel or synergy peel ‘MAST’) for 20 min. The skin samples were harvested on Day 7 to quantify melanin (histochemical imaging) and gene markers of melanin synthesis (RT-qPCR). We followed our previously published method for histochemical imaging and image processing to visualize and quantify melanin. For gene markers, biopsies were mechanically disrupted and homogenized, RNA was isolated (RNeasy Plus 96 kit from Qiagen, Inc. USA) and 100 ng of extracted RNA was used to synthesize cDNA (cDNA reverse transcription Kit from Applied Biosystems, USA). RT-qPCR was done using QuantStudio™ 5 real-time PCR (Applied Biosystems, USA) and the three gene markers of melanin synthesis (TYR, TYRP1 and DCT) were quantified using TaqMan real-time qPCR assays (Applied Biosystems, USA). For the gene expression study, TenSkin™ model from Ten Bio, Ltd., United
Kingdom was used to grow skin explants under optimum mechanical tension to mimic the real in vivo environment.27

2.4 | Subject recruitment and facial peeling performed by US board-certified dermatologist

Nine subjects representing different ethnicities, Fitzpatrick skin types (I–V) with dark or hyperpigmented patches on the face (photodamage, melasma and acne-induced post-inflammatory hyperpigmentation) were recruited for the proof-of-concept testing (case study). Table S1 (Supplementary information) captures the demographic details of the subjects and Appendix S1 (supplementary information) lists the inclusion and exclusion criteria for subject recruitment. The study protocol was approved by the US Institutional Review Board, Inc. in Miami, FL (U.S.IRB2022/CP01). The signed informed consent was taken from the subjects before their participation in the study. MAST Peel was dispensed by a US board-certified dermatologist, Dr. Handler, following manufacturer’s guidelines for peeling, post-peel care and for tolerability assessment (Appendix S2, supplementary information). There were two peeling sessions, 4 weeks apart. See Table 1 for summary of proof-of-concept clinical study design including subject visits for peeling and measurements.

2.5 | VISIA-CR imaging and dermatologist’s scoring

There were three measurement sessions: before peel (Week 0), 4 weeks after first peel (Week 4) and 4 weeks after second peel (Week 8). Visia-CR 4.3.1 photography with special optics and RBX® image processing technology from Canfield Scientific, USA, to separate red from brown was used to capture and analyse images of the face from three angles (front, left and right).28,29 The study dermatologist assessed changes in facial global appearance (uneven pigmentation/skin tone, skin texture, fine lines/wrinkles and redness) using a 5-point scale (0 = No, 1 = minimal, 2 = mild, 3 = moderate and 4 = severe).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Summary of case study design (N=9 participants).</th>
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<tr>
<td><strong>Procedure</strong></td>
<td><strong>Intervention/study week</strong></td>
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<td></td>
<td><strong>Screening</strong></td>
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<td>Informed consent</td>
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<td>Subject self-assessment</td>
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<tr>
<td>Facial swabbing</td>
<td>X</td>
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<tr>
<td>Visual assessment by dermatologist</td>
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<tr>
<td>VISIA-CR imaging</td>
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<tr>
<td>VivaScope imaging</td>
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<tr>
<td>MPTflex imaging</td>
<td>X</td>
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<tr>
<td>Subject self-assessment (post-­toner application)</td>
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<tr>
<td>Chemical-peeling</td>
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2.6 | Non-invasive optical biopsy imaging

Optical biopsies of the facial skin were taken using VivaScope 1500 and MPTflex, as reported previously.30,31 Medical grade ECG rings were placed on regions of interest on the cheeks to monitor the same spot over the course of the clinical study (longitudinal imaging). Images of skin anatomy (keratinocytes, melanin, collagen, etc) were acquired following manufacturer’s protocol, using a tissue cap and conducting gel for VivaScope 1500 imaging (830 nm laser with maximum power 20 mW) and a magnetic ring with coverslip, immersion oil and water for MPTflex imaging (tunable laser at 760 and 890 nm with maximum power 39 mW). Dermoscope images were also acquired prior to imaging using vivascope 1500. VivaScope images were studied primarily to visualize melanin,30 while MPTflex images were used to visualize keratinocytes.31 Similar to the previous report,32 FLIM images and photon counts were acquired at two different wavelengths (760 and 890 nm) to accurately discriminate and quantitate the NADH from other autofluorophores in the skin like melanin and FAD. FLIM images were processed offline using SPCLimage software from Becker and Hickl GmbH, Germany, as reported previously.33

2.7 | Facial swabbing for microbiome analysis

Swabbing of the cheeks was performed following the procedure reported in previous report,34 but with some modifications. The special large contour FLOQswabs® from Copan Diagnostics, Inc. USA (Cat#528C) was used to enable faster collection and efficient extraction of the samples for DNA extraction. The entire cheek was swabbed after impregnating the applicator of the sterile swab in PBS. The cheek was swabbed for 30 s by dabbing in a rolling manner and in horizontal directions to ensure that the entire surface area of the cheek was covered. After the sample was collected, the tip of the swab was broken from breakpoint and placed in a sterile tube, stored immediately at −80°C until tested for microbiological information. 16S rRNA sequencing was done using the MiSeq device (Illumina) and the data were processed to obtain alpha and beta diversity indices, as reported previously.29,35 Further analysis was done on two most abundant species, P. acne and S. epidermidis to monitor changes before and after peel application.

3 | RESULTS

3.1 | Computational modelling revealed surprising findings on molecular interactions between depigmentation agents and hTYR

Molecular docking (Figure 1A, i) shows binding of some well-known peeling and depigmentation agents on the catalytic and allosteric sites of hTYR, enabling us to predict their binding affinity to inhibit hTYR (Figure 1A, ii). Ellagic acid (commonly found in pomegranate),
glabridin and glycyrrhizic acid (commonly found in licorice), butyl and ethyl derivatives of resorcinol and arbutin (a derivative of HQ) showed strongest inhibition with binding affinity < −6 kcal/mol, highlighted by Tier I (Figure 1A, ii). KA, AzA, HQ, tranexamic acid (TXA), ascorbic acid, mandelic acid and hexyl derivative of resorcinol show moderate activity (affinity between −4 and −6 kcal/mol, Tier II). Surprisingly, KA was found to bind to catalytic site (active) as well as allosteric site#1 (Figure 1A, i); however, binding to the allosteric site is more stable (Figure SI, and Videos SI and SII, supplementary information). KA forms two hydrogen bonds at active site, while three hydrogen bonds at the allosteric site, which could be the possible rationale behind higher binding stability at the allosteric site (Figure SI).

Mandelic acid binds to allosteric site while TXA binds to catalytic site (Figure 1A, i and 1B, i). Figure 1B shows the reaction

FIGURE 1 In silico computational biology (A, B), and in vitro assay (C) showing screening results to identify LMP-TXA synergistic technology to inhibit tyrosinase enzyme. Binding site (A, i) and binding affinity (A, ii) of different skin lightening agents using molecular docking. Mechanistic understanding behind synergistic effect between mandelic acid and TXA using molecular dynamic simulations (B). Synergistic effect between lactic (L), mandelic (M) and pyruvic (P) acids (C, i) and the synergy validation by CompuSyn (C, ii), green line means synergistic effect, thicker the line stronger the synergistic effect. TXA, tranexamic acid.
kinetics to elucidate interactions of TXA and mandelic acid with hTYR. That reveal the possible mechanism of synergistic action: a conformational change in mandelic acid at allosteric site (Figure 1B, ii) around ~50 ns of simulated reaction lead to the stabilize of the hTYR structure in presence of TXA that has stable binding to catalytic site (Figure 1B, iii). The inhibition of hTYR by TXA is comparable to KA but stronger than HQ with binding affinity −5.42 versus −5.36 versus −5.13 kcal/mol, respectively (Table 2). This could be because TXA and KA form three hydrogen bonds with the enzyme (vs HQ that form two bonds), and compared to KA, TXA binds stably to copper in the catalytic site (Table 2, Figure SII). TXA is stable inhibitor of the enzyme as compared to HQ, which was not stable (Videos SIII and SIV, supplementary information). The unstable binding of HQ to hTYR could be because HQ is reported to act as substrate as well as inhibitor.36

The strongest triple combination synergy effect between alpha (lactic and mandelic acid) and keto acid (pyruvic acid) was identified (Figure 1C, i) showing ~10 times higher anti-TYR activity than their additive effect (21.9% vs. 2.5%). Among all mixtures, lactic and mandelic mixtures show strongest in vitro synergistic effect (data not shown), which was consistent with CompuSyn results (Figure 1C, ii), thickness of the green line denotes strength of the synergy.

3.2 | After single application, MAST peel is superior to commercial HQ-KA peel in inhibiting melanin

Figure 2 shows the melanin inhibition effect of MAST peel compared to commercial HQ peel that also contained KA (HQ-KA peel). There was no significant reduction in expression of TYRP1 and DCT genes after treatment with HQ-KA peel, but MAST peel (Figure 2A). Histochemical imaging showed similar results (Figure 2B), significant reduction in melanin intensity after MAST peel (~17%) but no change after HQ-KA peel (~1%). Figure 2C–E shows representative histochemical images used for quantification of melanin. The skin retained its structural integrity after peeling and histochemical

<table>
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<th>Active ingredient (ligand)</th>
<th>Binding affinity (kcal/mol)</th>
<th>Inhibition constant (mM)</th>
<th>Number of H bonds</th>
<th>Bond with copper</th>
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<td>HQ</td>
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<td>0.119</td>
<td>2</td>
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</tr>
<tr>
<td>KA</td>
<td>−5.36</td>
<td>0.035</td>
<td>3</td>
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</tr>
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</table>

Abbreviations: HQ, hydroquinone; KA, kojic acid; TXA, tranexamic acid.

FIGURE 2  Ex vivo testing on human skin explants to compare melanin inhibition potential of MAST peel versus commercial peel containing HQ and KA. (A) Gene markers of melanin synthesis normalized to RPS13 content (N=4), and (B) melanin quantification from histochemical images (N=6). Representative histochemical images of melanin are shown for untreated ‗control‘ (C) MAST peel (D) and HQ-KA peel (E). *Denotes significant difference versus untreated control (p < 0.05), ** denotes significant difference versus HQ-KA peel and % change is with respect to untreated (control). DCT, dopachrome tautomerase; Ed, epidermis; M, melanin; RPS13, ribosomal protein S13; SC, stratum corneum; TYR, tyrosinase, TYRP1, tyrosinase-related protein 1.
processing, which is evident through intactness of stratum corneum, epidermis and dermis (Figure 2C–E). Melanin, grey/black molecules in the histochemical images, was abundantly present at the basal layer (epidermis-dermis junction) and showed reduction or fading after MAST peeling.

3.3 | MAST peel shows clinical implications to manage hyperpigmentation without any downtime

All subjects (N=9) completed the study and there were no adverse events. No subjects experienced frosting, and only two subjects developed minor visible exfoliation, but not enough to avoid attending office and social gatherings (no downtime). A clear decrease in brown patches and redness was observed in most cases. Figure 3 shows before and after VISIA photographs to represent cases of photodamage (A, B), melasma (C, D), and acne-PIH (F) with improvement after two peeling sessions. A decrease in redness and brown spots is even more appreciated after processing of VISIA images using RBX® image processing to separate brown from red (Figure SII, supplementary information). The acne-induced PIH case reported a reaction to sunscreen and was corrected after replacing the sunscreen with a commercial sunscreen specially designed for oily and acne-prone skin. Figure 3F shows dermoscope and VivaScope images to reveal reduction of acne-induced PIH spots and the complete closure of all three comedones after peeling.

Figure 3E shows the average score calculated from the dermatologist’s objective visual assessment of each subject before and after chemical peeling. There is a global improvement in most, if not all subjects, with an average score improvement in uneven pigmentation/skin tone, skin texture, redness (Erythema) and fine lines/wrinkles. Three out of four cases of photodamage showed one to two step improvement in discoloration, all four cases of melasma showed improvement, but only by 0.5 points on the global appearance scoring scale. Decrease in redness was evident in the majority of the subjects, and more pronounced in melasma cases.

3.4 | Optical biopsy revealed underlying structural and functional changes in facial skin after peeling

For illustration purposes, Figure 4 shows non-invasive optical biopsy imaging instruments, VivaScope (Figure 4A) and MPTFlex (Figure 4B). The insets shows example of a participant with an ECG ring used for longitudinal imaging. After peeling, a decrease in visible superficial discolouration was observed under the dermoscope, which could be due to the underlying melanin reduction in the epidermis layer visualized by VivaScope (Figure 4C, i, ii). Melanin (small solid white dots) and melanin-containing keratinocytes (honeycomb structure) are visible under VivaScope; however, keratinocytes are even better appreciated under MPT-FLIM images (Figure 4D, i, ii). The quality of ultra-high resolution MPT images (Figure 4D) are comparable to what has been reported previously to image keratinocytes and quantify intracellular NADH incivivo on face31 and reconstructed human skin.32 There seems to be a noticeable increase in NADH intensity after peeling (black and white MPT images); however, it could be the interference from intracellular melanin, as highlighted by arrows in pseudo-coloured FLIM images in Figure 4D, i. In pseudo-coloured FLIM images, melanin is shown by red and NADH in fluorescent green. Using FLIM, we were also able to accurately discriminate between free NADH (~300 picosecond, Figure 4E, ii) and protein-bound NADH (~2300 picosecond, Figure 4E, iii), values are in agreement with previous reports using MPTFlex.33,37 There was a significant % decrease in lifetime of NADH before vs after peel (Figure 4E), free NADH, Tau 1 (Figure 4E, ii), protein bound NADH, Tau 2 (Figure 4E, iii) and mean lifetime ‘Tau M’ (Figure 4E, iv).

3.5 | Chemical peeling does not induces microbial dysbiosis

Owing to the virtue of special FLOQSwab® technology, all swab samples (9 × 3 subjects × time points) produced enough microbial yield to give more than 10000 reads, except one sample that produced 7342 reads (Figure SIV), Figure 5A shows the abundance of top 30 species found on cheek, and propionibacterium acne (14%–91% abundance) and staphylococcus epidermidis (0.5%–26% abundance) being most abundant, findings similar to previous report.35 S. aureus, typically related to infection and atopic dermatitis,36 was below the detection limit, except two subjects with <0.1% abundance. Although not significantly different, there was a noticeable change in species richness (Shannon diversity) after chemical peeling (Figure 5B), which is in agreement with increase in richness of operational taxonomic unit (Figure SIV, Supplementary Information). There was no structural difference (beta diversity) before and after peeling (Figure 5C). A decrease in P. acne after each peeling session can be observed in Figure 5A, and appreciated after quantification (Figure 5D). As expected the PIH case with acne showed P. acne abundance, but surprisingly it was >90% of total facial microbiome. VISIA-CR images of this acne-induced PIH case (Figure 5E), shows a decrease in Porphyrin, a fluorescent metabolite produced by P. acne.38,39

4 | DISCUSSION

Using in vitro enzyme assays and in silico computational biology, we were able to identify a synergistic mixture of four acids (Lactic, mandelic, pyruvic and traxenamic acids), aka MAST peel technology (Figure 1). Our in silico predictions using molecular docking (Figure 1B) were similar to in vitro results (data not shown), except in the case where ellagic acid and arbutin corresponded to lower activity in vitro. This variance could be explained by (1) the purity of arbutin and ellagic acid was not 100% in vitro experiments, (2) the source of tyrosinase enzyme was mushroom for in vitro assay.39
Using MD, KA was found to have affinity to bind to catalytic as well as allosteric site (Figure 1A), however, MDS revealed that binding of KA to allosteric site is more stable than catalytic site (Figure S1, and Videos S1 and SII). The strong binding of KA to the allosteric site is in agreement with another contradictory finding, that opposes classical KA binding theory to the copper in the catalytic site. Similar
observations were made for HQ. HQ binds strongly to the catalytic site but binding was unstable (Video SIII). Similar findings on instability of the HQ binding with tyrosinase enzyme has been reported previously and it could be because HQ acts as a substrate as well as inhibitor of tyrosinase enzyme. We believe the unstable interactions could be the reason why HQ is associated with refractory melasma.

Compared to HQ, we found that TXA was a strong and stable inhibitor of tyrosinase (Table 2, and Video SIV), hence it was identified as potential replacement to HQ. In the last couple years, TXA has emerged as a well-tolerated effective alternative to HQ in treating refractory melasma.

The multi-pronged synergistic action of our MAST peel design is supported by a deceptive simple solution identified by plastic surgeons to combine peeling acid with depigmentation agent to target complex pathology of refractory melasma. The peeling acids (lactic, mandelic, and pyruvic acids) induce an effect, thereby facilitating TXA penetration across the skin barrier. Usually, a 4% TXA peel/solution is combined with microneedling to enhance delivery of TXA and achieve better clinical outcomes. However, we got good results with low level of TXA (1% in MAST peel), which could be because of 1) penetration enhancement due to the peel’s keratolytic effect, and 2) Synergy between TXA and mandelic acid to target tyrosinase enzyme from two different sites (Figure 1C). Using MDS, we were able to elucidate the mechanism of synergistic action between TXA and mandelic acid: TXA binding to catalytic site of the tyrosinase enzyme induced conformational change in the mandelic acid
BHARDWAJ et al. (Figure 1C, ii), which lead to a stable binding (Figure 1C, iii). Synergy could be the rationale behind our surprising superior performance of MAST peel over a commercial peel containing HQ and KA (Figure 2). Significant inhibition of melanin after a single peel is a significant advantage of MAST over commercial HQ-KA peel (Figure 2A,B), because prolonged use of HQ and KA is associated with skin irritation, erythema, ochronosis, contact dermatitis and sunburns.5,41

Another evidence of synergy in action may be stipulated from clinical outcomes after MAST peeling. A decrease in hyperpigmentation and surprisingly also in erythema was observed in most of the cases recruited for the study (Figures 3A-D and SIII). A decrease in erythema may be attributed to (1) TXA, a tyrosinase and plasminogen inhibitor whose multi-pronged action can target melanogenesis as well as angiogenesis,42–44 (2) synergistic action between our mild peeling acids and (3) less acidic, because MAST peel is buffered to pH 3.6 to bring the pH closer to skin’s physiological pH 4.5 and hence expect little to no inflammatory response.45 All subjects were able to tolerate maximally applied two layers of the peel and completed both peeling sessions. Unlike TCA peels, there was no frosting and exfoliation was minimal (only noticed by two subjects). Participants with skin of colour, for example acne-induced PIH case in Figures 3E and 5E (Fitzpatrick V, African-American) were able to tolerate and show improvement after MAST peel. Although the sample size of our proof-of-concept clinical study was small (N=9 subjects), based on dermatologist’s experience in peeling and the fair representation of people from different ethnicities and Fitzpatrick skin type I–V (Table S1), it is reasonable to suggest that the MAST peel may be well tolerated by all skin types.

By using VivaScope® and MPTflex™, we were able to successfully visualize and monitor real-time changes in skin anatomy after peeling (Figure 4). There were no obvious signs of inflammation or inflammatory cells after MAST peeling, unlike our previous study on TCA peel that induces temporary inflammation.13 After MAST peel, a decrease in melanin content ex vivo (Figure 2D) and in vivo (Figure 4C) could be the underlying mechanism behind visible surface improvement in discoloration imaged by VISIA-CR (Figure 3A–D), dermoscope (Figure 4C) and dermatologist’s visual assessment (Figure 3E). Using the VivaScope we were able
to monitor acne-induced PIH microenvironment. VivaScope was able to visualize three invisible comedones/papules at the site of acne-induced PIH spots, which were otherwise not visible to dermatologist’s eye and even after using a dermoscope with 15x optical zoom (Figure 3F). An improvement in acne/acne-induced PIH case after MAST peel (Figure 3F) may be attributed to pyruvic...
acid and mandelic acid, which are clinically reported to be well tolerated and effective alternatives to azelaic acid and salicylic acid peels to treat acne. 46-48 Due to resolution limitations of the VivaScope, it is difficult to decisively label the surrounding highly reflective organelles around the open comedones (before peel) as inflammatory cells or melanin. Using ultra-high resolution MPTflex, we were able to visualize keratinocytes as well as melanin containing keratinocytes, which were otherwise poorly visible under VivaScope (Figure 4). Although quality of our MPTflex images are comparable to previous reports using MPT to visualize and quantify NADH, 31 we did not use their approach to quantify NADH because there are other molecules in skin that autofluoresce when excited at wavelength ~760 nm (NADH, FAD, Melanin). Therefore we used two laser wavelengths (760 and 890 nm) to discriminate between aforementioned fluorophores, and acquired their fluorescence lifetime information using FLIM detector to accurately discriminate (Figure 4D) and quantify NADH (Figure 4E). 32, 33 The decrease in lifetime of the intracellular NADH after peeling (Figure 4E) indicates increase in metabolic activity of the keratinocytes. Similar findings on decrease in NADH lifetime have been correlated with healing of wounded skin, 37, 49 and recovery of UV-damaged skin. 32 Skin aging (chronological and UV-induced), dyspigmentation and other skin health conditions/diseases have been linked to mitochondria dysfunction, which may be treated by boosting energy of the mitochondria. 50

After MAST peel, we did not find any significant change in microbiome, but a trend showing increase in species diversity and decrease in P. acne (Figure 5D). However, it was a small proof-of-concept study (N = 9), and no conclusion can be drawn on the dispusive potential of the MAST technology on microbiome. Considering chemical peel is a popular intervention for management of acne, melasma, etc. our findings set foundation for further research in peels and post-peel regimens to intervene microbiome in patients with acne (P.acne/C.acne phylotypes), atopic dermatitis (S. aureus), melasma (Actinomyces, Collinsella) etc. 34, 51-53

Although we were able to record real-time the blood flow in the capillaries (Video SV, supplementary information), detailed analysis and models need to be developed to make any conclusion on changes in blood volume and dynamics before and after peeling. It will be particularly important for melasma, acne, PIH and other sensitive and inflammatory skin conditions like psoriasis, atopic dermatitis, Rosacea, etc., where redness and inflammation are salient features. Using powerful features of ultrafast in situ VivaScope imaging (turnaround time <1 min) we can get rich anatomical information on skin health. Using this information, a trained dermatologist/professional can take pathology-driven real-time decisions to personalize treatment for better outcomes. For example, by using VivaScope as a guide to understand melanin distribution in different skin layers, dermatologists and surgeons can make a better choice in selecting a peel (ingredients, depth, layers, application pressure, etc.) and/or their synergistic combination with laser, microneedling, etc. to treat different types of HP, such as epidermal melasma versus dermal melasma.

5 | CONCLUSIONS

Owing to the synergistic action of the proprietary multi-acid blend, the MAST peel when administered by an experienced professional showed strong: (1) ex vivo anti-pigmentation activity on human skin explants as compared to a commercial professional peel of similar strength but containing HQ and KA, and (2) clinical efficacy with minimum downtime (no frosting and little visible exfoliation) to effectively manage common hyperpigmentation conditions like melasma, photo-damage and acne-induced PIH. The MAST peel induced no dysbiosis of the facial microbiome and showed good tolerance by all people who participated in the study. However, it was small proof-of-concept clinical study (N = 9) and further investigation is required to conclude safety and efficacy on people with skin of colour and sensitive skin.

Using computational biology and optical biopsy, we were able to gain new insights into the pathophysiology of skin after chemical peel. The visible improvement in dull, hyperpigmented and acne could be correlated to invisible histological changes in intracellular NADH (energy marker in mitochondria of keratinocytes), melanin and comedones visualized real-time by non-invasive optical biopsy using MPTflex™ and VivaScope®. Based on our mechanistic findings from computational biology (TXA vs. HQ), performance testing on skin explants (MAST peel with TXA vs. commercial peel with HQ), and a comprehensive review of 46 articles on comparing TXA versus HQ for melasma treatment, 5 we can conclude that TXA is a promising alternative to HQ.

AUTHOR CONTRIBUTIONS

Conceptualization, Vinay Bhardwaj and Junhong Mao; Data curation, Vinay Bhardwaj, Marc Zachary Handler, Chloe Azadegan and Pritam Panda; Investigation, Vinay Bhardwaj and Marc Zachary Handler; Methodology, Vinay Bhardwaj, Chloe Azadegan, Marc Zachary Handler, Hans Georg Breunig; Software, Vinay Bhardwaj and Isabella Wenskus; Supervision, Vinay Bhardwaj, Junhong Mao and Isabel Diaz; Writing—original draft, Vinay Bhardwaj; Writing—Editing, Marc Zachary Handler, Karsten Koenig, Isabel Diaz, Writing—review, All co-authors. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.
INSTITUTIONAL REVIEW BOARD STATEMENT
The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the US Institutional Review Board, Inc. in Miami, FL (U.S. IRB2022/CP01).

INFORMED CONSENT STATEMENT
Informed consent was obtained from all subjects involved in the study before participating in any study-related activities. Written informed consent was obtained from the patients to use their personal information (including microbiome data and photos) for the manuscript publication.

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REFERENCES


SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Video S1: Kojic acid binding to active (catalytic) site of human tyrosinase enzyme.
Video SII: Kojic acid binding to allosteric site of human tyrosinase enzyme.
Video SIII: MD simulation showing binding of HQ to human tyrosinase enzyme is not stable.
Video SIV: MD simulation showing TXA binding to human tyrosinase is stable.
Video SV: Real-time video captured from VivaScope to visualize blood flow in facial skin.

Appendix S1: Inclusion and exclusion criteria.
Appendix S2: Peeling procedure.