Matrixyl Patch vs Matrixyl Cream: A Comparative In Vivo Investigation of Matrixyl (MTI) Effect on Wound Healing

ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Matrixyl Patch vs Matrixyl Cream: A Comparative In Vivo Investigation of Matrixyl (MTI) Effect on Wound Healing

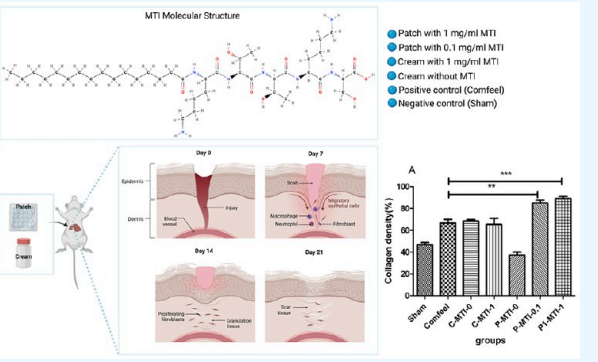
Maryam Kachooeian, Zahra Mousivand, Elham Sharifikolouei, Mehrnoosh Shirangi, Loghman Firoozpour, Mohammad Raoufi,* and Mohammad Sharifzadeh*

Cite This: *ACS Omega* 2022, 7, 24695−24704

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Wound healing is one of the most complex biological processes. Studies show that Matrixyl (MTI), known as a cosmetic peptide, can lead to a faster healing process. The contribution of MTI to collagen formation during wound healing also depends on its mode of delivery and its release over time. Here, we investigate two modes of MTI-delivery system, the influence of MTI patch for wound healing application in comparison with MTI cream. In this study, animals were randomly divided into seven groups and studied for 21 days: patches containing two different concentrations of MTI (P-MTI-0.1 mg and P-MTI-1 mg), a cream containing MTI (C-MTI-1 mg), a patch (P-MTI-0), a cream with no MTI (C-MTI-0), a positive control (Comfeel), and a negative control (sham) group. To study the wound healing process, the change in collagen density, angiogenesis, epitheliogenesis, histopathology, immunohistochemical analysis, and wound area through imaging was monitored and measured. The macroscopic results showed that wound healing was improved from 63.5 up to 81.81% in treatment groups compared to that in the negative control group ($P < 0.05$ and $P < 0.001$). In addition, C-MTI-1 and P-MTI-1 had a larger impact on wound healing compared to that in the positive control group (Comfeel, $P < 0.05$). In hematoxylin and eosin (H&E) staining analysis, the rejuvenation of skin appendage was visible in both groups of cream and patches with MTI. According to the obtained results, the re-epithelialization had a higher range for the patch with MTI in comparison with cream containing MTI and positive control.



1. INTRODUCTION

Skin, as the largest organ in the body, encloses the organs, tissues, and bodily fluids and is thought to be an effective barrier against external invasions.[1] The epidermis, dermis, and hypodermis are the three main layers of skin. Skin layers could lose their natural cohesion due to a variety of factors including cuts, physical damage, and burns. This might result in a type of tissue rupture (wound) called skin ulcers.[2] Created wounds could be acute or chronic (also known as nonhealing wounds).[3] The wound healing process is a complex and relatively long process that is initiated by an immune response to the wound, and it continues until the damaged tissue is completely repaired.[4] This process involves both cellular and molecular events. Therefore, after skin damage, inflammatory responses take place and the process of the production of new collagen by dermal cells begins, leading to a new covering tissue formation in the epidermis.

Peptides are compounds formed by the binding of several amino acids, through the binding of the carboxyl group of amino acid with the amino group of another amino acid, and the properties of these substances mainly depend on the amino acid sequence. In recent years, the use of peptide medications in the pharmaceutical industry, and in particular for wound healing applications, has been significantly increased due to their unique properties such as high propensity and specificity, quick and easy synthesis, small size, good permeability, low drug interaction, labeling ability, and less tissue accumulation.[5] In fact, the peptides in skincare products offer an assortment of benefits including antiaging and sun protection to fasten mending of wounds.[6] Numerous kinds of research have reported peptides' benefits in reducing fine lines and wrinkles, tightening the skin, reducing the size of open skin pores, reducing skin inflammation, protecting against sun damage, and accelerating wound healing.[7]

Matrixyl (MTI) is a palmitoyl-pentapeptide, also known as a peptide. It is a new compound that has a profound influence on collagen formation.[8] It contains five amino acids (Lys-Thr-Thr-Lys-Ser-OH or KTTKS-OH) that bind to a 16-carbon aliphatic chain at the N-terminus, and its molecular structure is shown in Figure 1. The aliphatic end increases the drug's entry into the

Received: April 26, 2022
Accepted: June 15, 2022

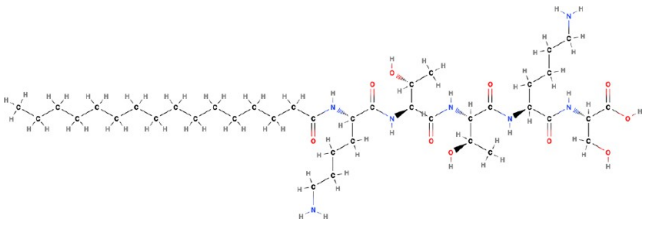


Figure 1. Molecular structure of MTI with Pal-Lys-Thr-Thr-Lys-Ser-OH.

skin's sebum structure and its impact by increasing the compound's lipophilicity. Typically, the problem of passing through the skin in topical products is due to the ionic nature of some compounds, which, as previously stated, is solved in Matrixyl by adding palmethoyl.[9]

In vitro studies indicate that MTI, palmitoyl-pentapeptide, is one of the signal peptides. It has been discussed that the mechanism by which MTI promotes collagen formation is based on the peptide reaching the fibroblast in the dermis after passing through the skin and stratum corneum. The peptide binds to the cell surface receptor, which is linked to a transducer, and data is transmitted to the cell via various signaling pathways. These pathways are similar to the TGF-β pathway since they all lead to increased collagen production. Furthermore, MTI promotes cellular processes such as collagenase inhibition to prevent collagen degradation, which eventually increases extracellular matrix protein production.[10,11]

Lintner et al. have demonstrated that using MTI as cream has a significant effect on increasing the collagen in the dermis and on reducing wrinkles.[12] Another study was conducted in 2016 to evaluate the effect of palmitoyl-pentapeptide (Pal-KTTKS) on wound contractility in relation to connective tissue growth factor and smooth muscle actin expression and showed a balance between the wound healing properties and profibrotic abilities of pentapeptide KTTKS. Therefore, it was suggested to consider palmitoyl-pentapeptide as a therapeutic agent in the prevention of scar lesions.[13]

Skin patches are also one of the most recent shapes of skin products. Cosmetic patches are a unique skin delivery system that supplies the body's demand for ingredients and allows the transdermal transfer of active compounds.[14] Today, skin patches are widely used for various drug delivery purposes such as helping to quit smoking, reducing pain, controlling nausea, and treating hormonal disorders, and as contraceptives in women.[15] However, their utilization as skin patches is still limited and has several obstacles due to restrictions on the selection of prescription drugs from the skin pathway and the variability of skin absorption of the drug in different people.[16]

To date, no comprehensive study has compared the impact of patch peptides on wound healing compared to the common peptide cream. In this regard, we have synthesized Matrixyl, analyzed different parameters including epithelialization counting and collagen union and histological studies, and inspected whether the use of the patch form was especially superior to the use of the cream.

2. MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich Chemicals.

2.1. Synthesis of Matrixyl. The peptide was prepared through solid-phase synthesis based on the Fmoc strategy.[17] Fmoc (fluorenylmethyloxycarbonyl) was first introduced as an amino acid protector in 1970. In this strategy, the first amino acid has an amino acid moiety protected by Fmoc. This group is unstable under alkaline conditions and separates from the amino acid. 2-Chlorotrityl chloride (2-CTC) resin was used for

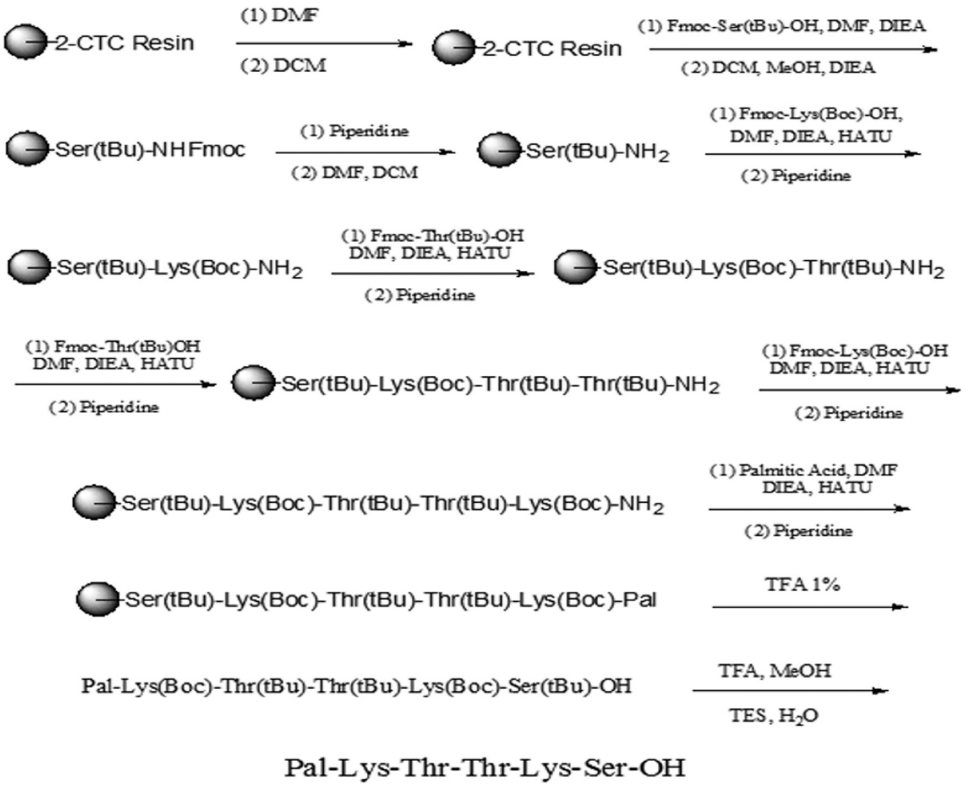


Figure 2. Schematic representation of the Matrixyl synthesis process.

https://doi.org/10.1021/acsomega.2c02592
ACS Omega 2022, 7, 24695−24704

Table 1. Summary of the Studied Groups

groups	C-MTI-0	C-MTI-1	P-MTI-0.1	P-MTI-1	P-MTI-0	Comfeel	sham
MTI concentration (mg/mL)	0	1	0.1	1	0	0	0

Matrixyl synthesis. The synthesis involved loading of C-terminal amino acid to resin; deprotection, i.e., the removal of N-terminal protecting group (PG) at amino residue; activation of the next amino acid at carboxy residue; coupling reaction; and finally, repeating this cycle until crude peptide was cleaved off the resin. To ensure amino acid coupling to the peptide−resin chain, the Kaiser test was used. The schematic representation of the Matrixyl synthesis process is presented in Figure 2. The validity of the synthesis was controlled by the Kaiser test, and the protocol is explained in the Supporting Information.

2.2. Evaluation of Synthesized Matrixyl (Pal-KTTKS). The mass spectrometer equipped with a Turbo ion spray source (Agilent, 641 QQQ) was used to identify and confirm the synthesized peptide structure (presented in Figure S1). Assay and purity of peptide were measured using high-performance liquid chromatography (HPLC) (Agilent 1200 instrument). The assay of the synthesized sample was compared with a white powder of Matrixyl (CAS No. 214047-00-4) purchased from Sigma-Aldrich (presented in Figure S2 and Table S1). Likewise, the purity of MTI was measured by HPLC and compared to that of distilled water, which was used as a blank (see Figure S3 and Table S2). Residual solvents after synthesis were measured by headspace gas chromatography equipped with a flame ionization detector based on USP ⟨467⟩ (Figure S4). The presence of heavy metals was assessed via calorimetry. The pH of the synthesized MTI was also measured at 1% w/w in distilled water by a pH meter (Table S3), and the moisture content was measured by Karl Fischer (Table S4).

2.3. Patch Formulation and Fabrication. Biopolymeric solutions containing hyaluronic acid were prepared at the concentration of 0.36% w/v in the deionized water. The solution was first mixed by a vortex for 1 h, followed by another hour of mixing on a hot stirrer (400 rpm). Thereafter, the solution was filtered by a 0.22 μm membrane to remove undissolved fragments until a very clear solution was obtained. An optimized 400 nm pore size ceramic membrane was selected to achieve the required skin properties of the patch and was mounted under the setup with an O-ring. Moreover, the extrusion process was conducted under a controlled temperature (37−39 °C) to facilitate the evaporation of the solvents from an extruded nanofiber. The fed solution was passed through the membrane by a syringe pump (Standard PHD ULTRA CP 4400, Harvard) at a constant flow rate of 200 μL/min. Matrixyl (MIT) was added in the concentrations of 5×10^{-5}% w/v and 5×10^{-4}% w/v. In addition, biopolymeric solutions were dried at 40 °C in a culture hood for 10 h.[18]

2.4. In Vivo Assessment of Wound Healing Activity. A total of 42 male albino Wistar rats weighing 200−250 g were used (6 in each group). The rats were kept in standard vivarium conditions (60% relative humidity and a temperature of 25 °C) and fed standard laboratory food and water. The experiment was approved by the Tehran University of Medical Sciences' animal ethics guidelines. The animals were shaved at the dorsal part with intraperitoneal injections of ketamine (90 mg/kg) and xylazine (10 mg/kg) under anesthesia. On the dorsal area, a large wound was inflicted. Comfeel, a ready-to-wear dressing available in the market, was used as a positive control group. It consists of a semipermeable polyurethane film coated with a flexible, cross-linked adhesive containing sodium carboxymethylcellulose and calcium alginate as the principal absorbent and gel-forming agents. Sham was used as a negative control group. Generally, a sham was defined as a group of animals that are not exposed to any variable in the study, and in this case, their wounds were only bandaged without any further treatments. The animals were kept in separate cages and divided into seven groups: positive control (Comfeel), negative control (sham), P-MTI-0 (patch without any active substance), P-MTI-0.1 (0.1 mg of Matrixyl loaded on the patch), P-MTI-1 (1 mg of Matrixyl loaded on the patch), and C-MTI-1 (1 mg of Matrixyl in formulated cream), and C-MTI-0 (cream base). For 21 days, a layer of cream and patches was applied to wounds in different groups every 24 h. The wound healing process was observed and recorded by a camera (Canon EOS 4000D) using a ruler as a scale. ImageJ (version 1.46) software was used to calculate the wound reduction percentage of the wound area.

2.5. Histopathological and Immunohistochemical Analysis. On days 7, 14, and 21 postsurgery, the animals were sacrificed, and the scar tissues were harvested with a 0.5 cm margin of surrounding unwounded tissue. Half of each sample was immediately fixed in 10% neutral buffered formalin (pH 7.26) for 48 h and dehydrated, embedded in paraffin, and sectioned into 5 mm thickness. The dehydration process was based on the use of ethanol with gradually increasing concentration; therefore, samples were first placed in ethanol 70% and then transferred to ethanol 80, 90, and 99.999% respectively. Finally, the sections were stained with hematoxylin and eosin (H&E) and Masson trichrome (MT). The samples were further coded and an independent pathologist evaluated the slides using light microscopy (Olympus BX51; Olympus, Tokyo, Japan) to ensure a nonbiased evaluation.

2.5.1. Angiogenesis and Collagen Density. The number of blood vessels per slide in five microscopic fields (HPFs) was randomly counted and the average for three replications was reported. The percentage of collagen tissues in five microscopic fields with 100× magnification that were randomly selected was calculated using Image Pro-Plus software (version 6) based on the difference in color density and the average for three replications was reported.

2.6. Statistical Analysis. All results were compared by posthoc Newman−Keuls multiple comparison test using a one-way ANOVA analysis. Results with P values less than 0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism, version 7.

3. RESULTS

Patches were synthesized as described previously in Section 2.3. To confirm the successful synthesis of Matrixyl, its structure was detected and confirmed using mass spectroscopy (Figure S1). The assay of the peptide was evaluated based on the anhydrous method, and the value of 100.7% was calculated for it (Figure S2 and Table S1). Additionally, the purity of peptide was evaluated through HPLC analysis and estimated to be 97% (see Figure S3 and Table S2).

The wound healing process for different groups of the study, as summarized in Table 1, was compared on days 0, 7, 14, and 21 and are shown in Figure 3. According to this figure, from day 0 to

https://doi.org/10.1021/acsomega.2c02592
ACS Omega 2022, 7, 24695−24704

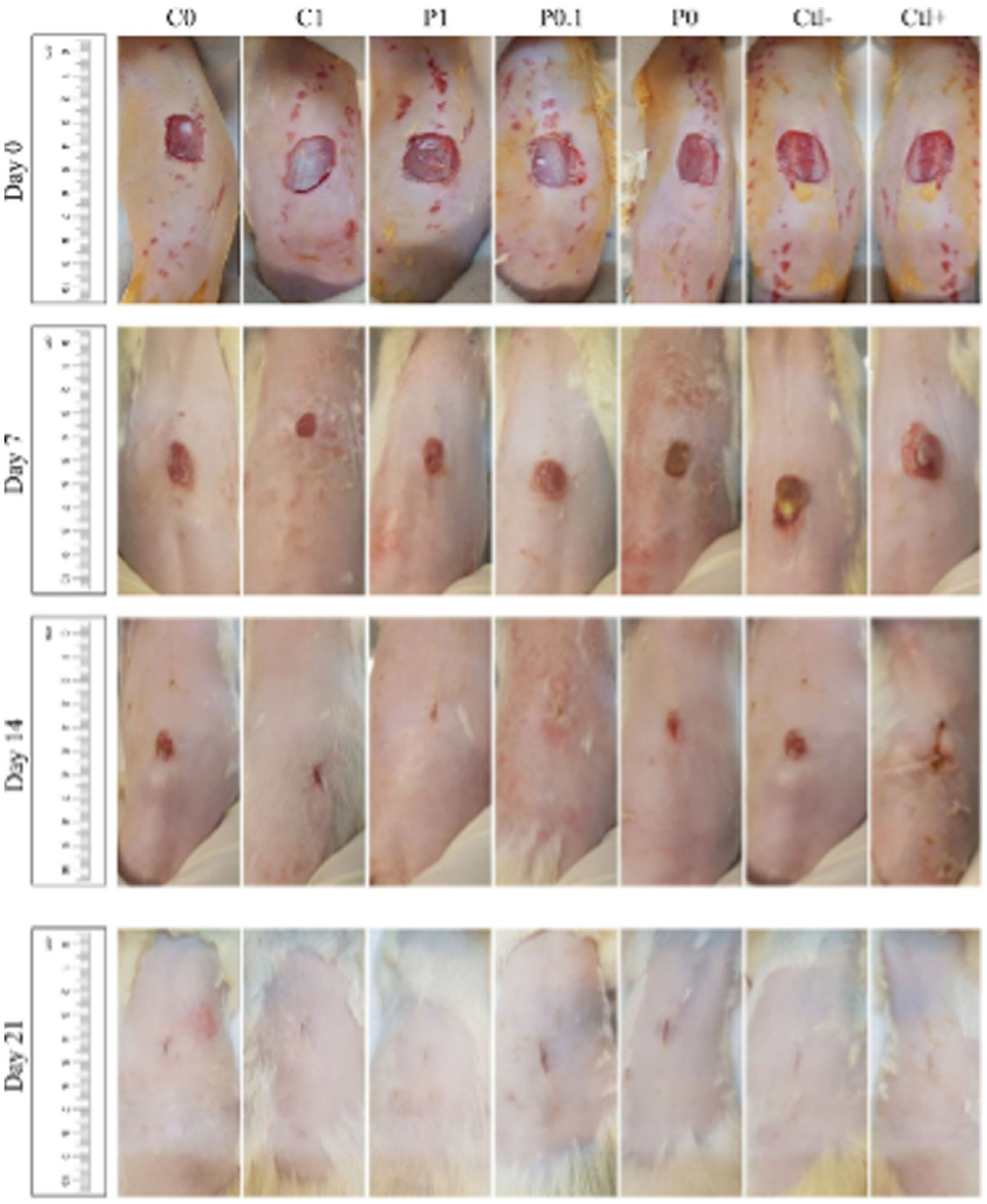


Figure 3. Macroscopic images of the induced wound on the dorsal area of the rat on days 0, 7, 14, and 21 in the studied groups: C-MTI-0 (C0), C-MTI-1 (C1), P-MTI-1 (P1), P-MTI-0.1 (P0.1), P-MTI-0 (P0), sham negative control, and Comfeel positive control.

7, no specific difference between defined groups was observed. On the 14th day, however, P-MTI-1 (patch with a high concentration of MTI), P-MTI-0.1 (patch with a low concentration of MTI), and C-MTI-1 had more alterations than sham and P-MTI-0 (patch without MTI). Nevertheless, on the final day of the study, almost all groups showed good restorative benefits. This observation was further studied quantitatively for the change in the wound healing area, and its analysis is shown in Figure 4. The full values are also presented in Table 2.

According to Figure 4A, at the end of the first week (day 7), the wound area reduction in all defined groups is comparable; meanwhile, going to Figure 4B, there is a significant wound area reduction ($P < 0.001$) in all groups that contain MTI in comparison with the negative control group (sham). Indeed, the most significant wound closure for the defined group in comparison with the negative control group is observed in the last week of the study ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$) (Figure 4C). This could indicate that the effectiveness of the defined groups with active compounds (MTI) does not decrease over time. Furthermore, it could be noted that the results from the patch with a low concentration of MTI (P-MTI-0.1) showed similar effects to those of the positive control group (Comfeel). On the other hand, wound contraction with Matrixyl cream and

https://doi.org/10.1021/acsomega.2c02592
ACS Omega 2022, 7, 24695−24704

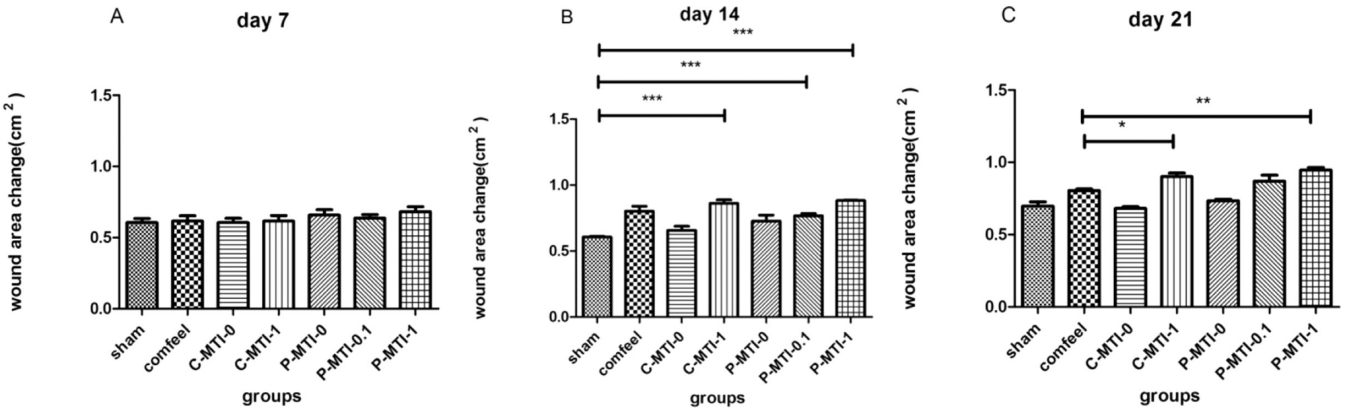


Figure 4. Quantitative analysis of wound area reduction in C-MTI-0, C-MTI-1, P-MTI-1, P-MTI-0.1, P-MTI-0, sham negative control, and Comfeel positive control on (A) day 7, (B) day 14, and (C) day 21. *P < 0.05, **P < 0.01, and ***P < 0.001.

Table 2. Comparison of Wound Area Change (%) in Treatment Groups on Days 7, 14, and 21[a]

groups/days	day 7	day 14	day 21
C-MTI-0 (C0)	60.51 ± 6.80	65.74 ± 6.01	68.06 ± 2.26^{*X_b}
C-MTI-1 (C1)	61.53 ± 8.78	86.12 ± 5.41$^{\#X_a}$	90.03 ± 4.42$^{\#X_a,*X_b}$
P-MTI-0 (P0)	65.87 ± 8.17	72.73 ± 8.98	73.411 ± 1.79
P-MTI-0.1 (P0.1)	63.73 ± 5.37	76.63 ± 3.56^{**X_a}	86.96 ±7.07$^{\#X_a}$
P-MTI-1 (P1)	68.24 ± 7.76	88.30 ± 0.43$^{\#X_a}$	94.71 ± 3.04$^{\#\#X_a,***X_b}$
sham (ctrl−)	60.51 ± 6.80	60.69 ± 0.90	69.72 ± 5.17
Comfeel (ctrl+)	61.58 ± 8.38	80.23 ± 7.41	80.39 ± 2.08

[a]Values are expressed as mean ± standard deviation for each group. Significant differences: *P < 0.05, **P < 0.01, and ***P < 0.001. X_a: in comparison to negative control, X_b: in comparison to positive control.

patch with a high concentration of the MTI (C-MTI-1 and P-MTI-1) was significantly higher than the positive control group (Comfeel) (*P < 0.05).

Histology analysis studies the structure and function of tissues and their components, and it is a suitable method for evaluating the effectiveness of various forms of pharmaceutical components and cosmetics. In this work, histological analysis of the skin wounds was performed by H&E staining on days 7, 14, and 21. In these images, magnifications of 40 and 100 were utilized for coloring H&E. Histological analysis of the skin wounds on day 7 is shown in Figure 5. In this figure, the H&E staining crusty scab indicated the production of granulation tissue. According to this analysis, there was no significant difference among the studied groups at this time point. The histopathological evaluation of the positive control (Comfeel) showed severe infiltration of inflammatory cells into the wound area. On this day, epidermis and dermis started to form for the C-MTI-0 and C-MTI-1 treatment groups. The wound healing process depended on collagen synthesis. Therefore, to further investigate the effect of creams and patches on wound healing, sections of animal skin tissues were stained with Masson trichrome (MT) staining (marked as MT*100). This staining was used to recognize the progress of collagen synthesis during granulation tissue formation and matrix remodeling. Collagen fibers were stained blue-green in the MT staining method, in which the intensity of this color corresponded to the relative amount of deposited total collagen and reflects the advancement of collagen synthesis and remodeling. According to MT staining, the collagen production in all groups is comparable.

Moving to the H&E analysis on day 14, presented in Figure 6, we observed the same crusty scab in all groups as a sign of granulation tissue. Histological examination revealed that epithelialization, one of the most important wound healing processes, started on this day. Although a narrow layer of the epithelial cell was formed in the positive control group (Comfeel), inflammation was also observed for it. Furthermore, epidermal proliferation was observed for both low and high MTI concentrated patches (P-MTI-0.1, P-MTI-1). For P-MTI-0.1, the re-epithelialization and rete ridges were observed on the same date. The inflammatory response in the cream treatment groups, C-MTI-0 and C-MTI-1, was considerably decreased. The MT staining analysis on this date showed an increased collagen production and rete ridge in the MTI-containing groups and the positive control group (Comfeel).

Finally, moving to H&E analysis on day 21 (Figure 7), in the positive control group, the epithelialization process was completed, and the inflammatory cells were significantly reduced in comparison to the negative control at the same time point. Furthermore, the inflammatory response and granulation tissue were gradually decreased for both low and high concentrations of MTI patches (P-MTI-0.1, P-MTI-1). These treatment groups had more resemblance to normal skin compared to other defined groups due to the formation of the thin epidermis, the presence of normal rete ridges, and the rejuvenation of skin appendages. In this manner, the presence of MTI in these patches realized the advantageous impacts of the repairing process. On this day, various degrees of re-epithelialization were formed in all the replicates of cream without MTI and cream with MTI treatment groups (C-MTI-0, C-MTI-1). The MT staining on day 21 showed that there was a clear increase in the density of blue in the patch with high concentration and patch with a low concentration of MTI (P-MTI-0.1, P-MTI-1) and relatively less in the positive control groups (Comfeel) and the group with cream with Matrixyl (C-MTI-1). Finally, it was evident that collagen production in patch groups was significantly higher than that in the cream groups.

https://doi.org/10.1021/acsomega.2c02592
ACS Omega 2022, 7, 24695−24704

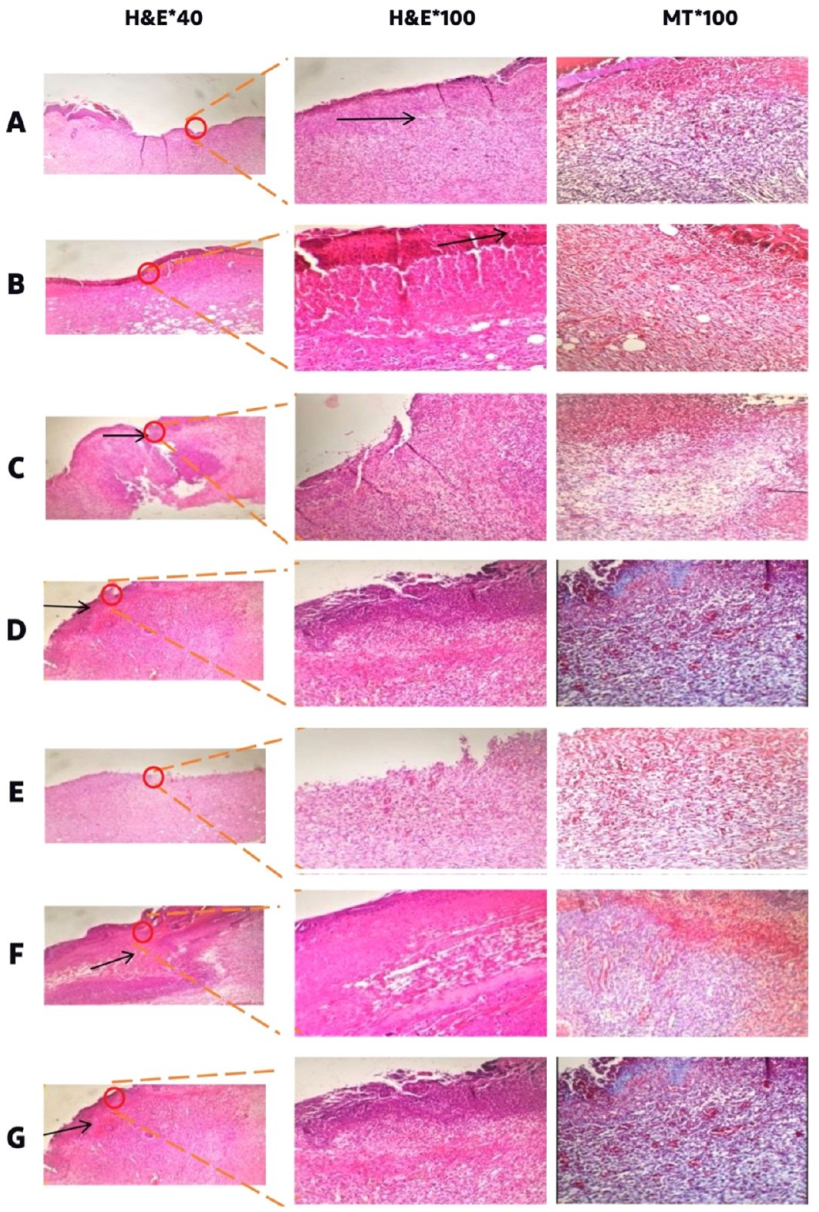


Figure 5. H&E and MT-stained microscopic sections of healed incisions in the different treatment groups on day 7 post-treatment, arrows: crusty scab. (A) C-MTI-0, (B) C-MTI-1, (C) P-MTI-1, (D) P-MTI-0.1, (E) P-MTI-0, (F) negative control, and (G) positive control.

The collagen density and angiogenesis quantitative analysis based on H&E figures were performed and are shown in Figure 8, and their exact values are reported in Table 3. Based on the angiogenesis analysis, the treatment response in the cream with MTI group was better than those of cream without MTI according to histomorphometric analysis. Based on the collagen density analysis, among all defined groups, the patch with a low concentration and the patch with a high concentration of MTI had the greatest collagen synthesis, followed by Comfeel. Hence, the positive effect of MTI on wound healing was quite evident. In contrast, the rate of collagen fiber synthesis and deposition in the wound area was the lowest in the negative control group. The appropriate angiogenesis had a Gaussian trend as reported before by DiPietro[19] and was observed in the following groups: P-MTI-0.1, P-MTI-1, C-MTI-0, C-MTI-1, and Comfeel. When the angiogenic process continued to increase until the end of the study, it was an unfavorable result, which was observed in the sham group and negative control studies. This inappropriate process was also evident in wound closure.

Epithelialization on day 21 was assessed semiquantitatively on a five-point scale: 0 (without new epithelialization), 1 (25%), 2 (50%), 3 (75%), and 4 (100%) (Table 4). All defined groups were compared with the sham group. Both patches with high and low concentrations of MTI had the highest rate of epithelialization compared to the negative control (***P < 0.001). Furthermore, the highest amount of efficacy was observed for the patch groups, the cream with Matrixyl group, and Comfeel group, respectively (**P < 0.01). However, the least amount of efficacy was observed for the sham group (*P < 0.05) (Table 4).

4. DISCUSSION

In this work, we successfully synthesized Matryxil (MTI), which was confirmed by mass spectroscopy, and investigated its effect on wound healing when utilized in the form of cream compared

https://doi.org/10.1021/acsomega.2c02592
ACS Omega 2022, 7, 24695−24704

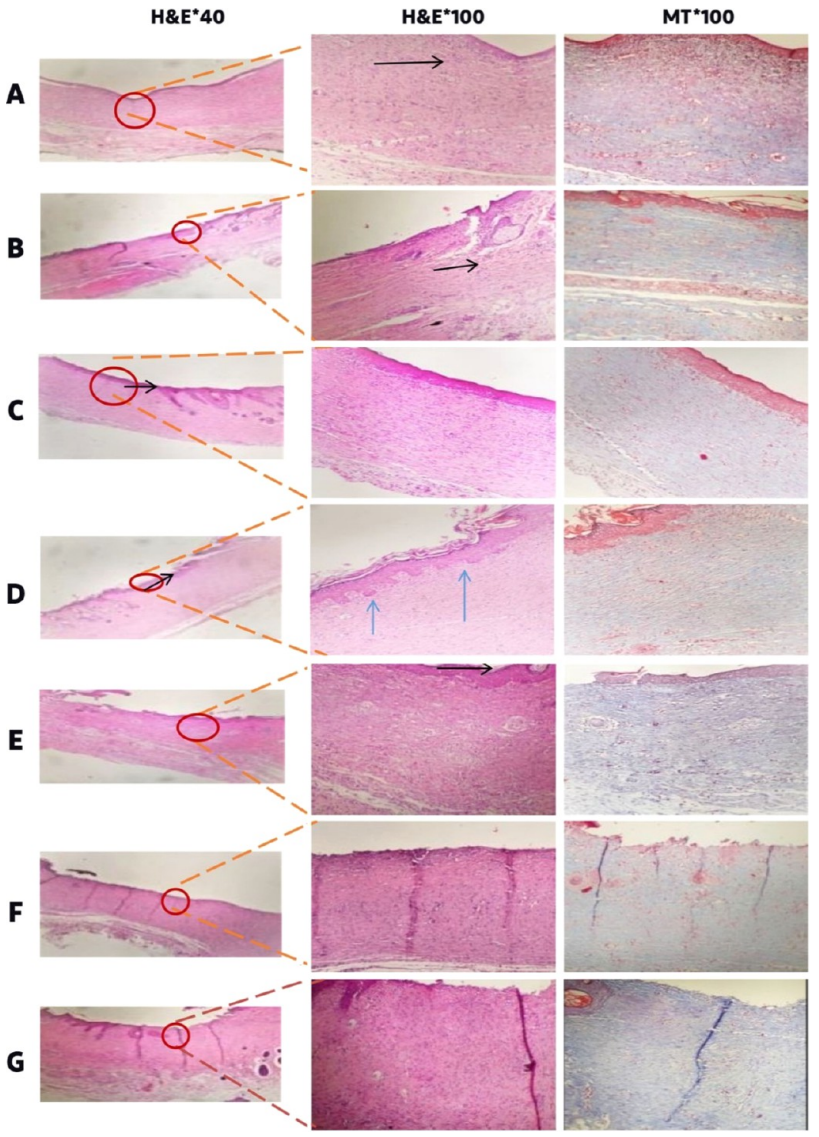


Figure 6. H&E and MT-stained microscopic sections of healed incisions in the different treatment groups on day 14 post-treatment. Black arrows: re-epithelialization; blue arrows: rete ridges. (A) C-MTI-0, (B) C-MTI-1, (C) P-MTI-1, (D) P-MTI-0.1, (E) P-MTI-0, (F) negative control, and (G) positive control.

to transdermal patches. We prepared seven groups of samples with different concentrations of MTI: positive control (Comfeel), negative control (sham), P-MTI-0 (patch without any active substance), P-MTI-0.1 (0.1 mg of Matrixyl loaded on the patch), P-MTI-1 (1 mg of Matrixyl loaded on the patch), C-MTI-1 (1 mg of Matrixyl in formulated cream), and C-MTI-0 (cream base), which were studied for their collagen density, angiogenesis, epitheliogenesis, histopathology, immunohistochemical analysis, and change in wound area.

The macroscopic analysis of the wound healing process indicated that a minimum 14-day post-treatment was required to observe a meaningful difference among the groups. On day 14, the effect and the contribution of MTI to wound healing were quantitively evident. Overall, these findings were in accordance with the findings reported by Jones et al., who studied the effect of C16-KTTKS amphiphile peptide on collagen stimulation in human fibroblasts and showed that the stimulation of collagen production was correlated with the concentration of the peptide.[11] In fact, both patches with low and high amounts of MTI, as well as cream with MTI showed a better wound closure compared with the positive control group. Another study conducted by Katayama et al. showed that the pentapeptide (KTTKS) was the minimum sequence necessary for the potent stimulation of collagen and fibronectin production in a variety of mesenchymal cells.[20] Indeed, this was the exact sequence present in Matrixyl. On day 21, the same trend was observed, showing the most promising results for cream and patch with 1 mg of MTI. In this analysis, even though the best result was observed for the Patch with 1 mg of MTI, no significant difference between the patch and the cream with the same concentration of MTI was detected.

The collagen formation in each group, however, had shown a different trend. In fact, both patches with high and low concentrations of MTI (P-MTI-0.1 and P-MTI-1) showed an improved collagen formation with 89 ± 3.6 and 85 ± 4.5% in comparison with C-MTI-1 (65.3 ± 9.6%). This means that the mode of delivery could impact collagen formation during the wound healing process.

Furthermore, H&E analysis showed that both patches with high and low concentrations of MTI had the highest rate of

https://doi.org/10.1021/acsomega.2c02592
ACS Omega 2022, 7, 24695−24704

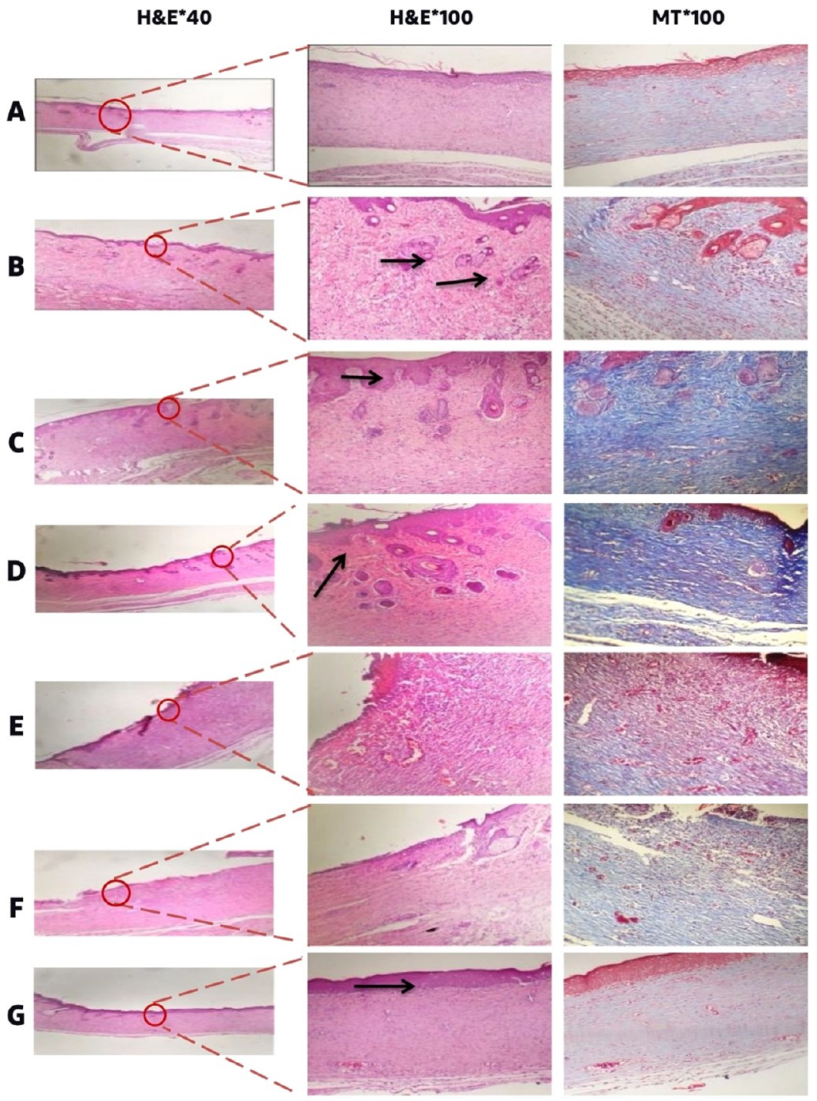


Figure 7. H&E- and MT-stained microscopic sections of healed incisions in the different treatment groups on day 21 post-treatment. Arrows: rejuvenation of skin appendages. (A) C-MTI-0, (B) C-MTI-1, (C) P-MTI-1, (D) P-MTI-0.1, (E) P-MTI-0, (F) negative control, and (G) positive control.

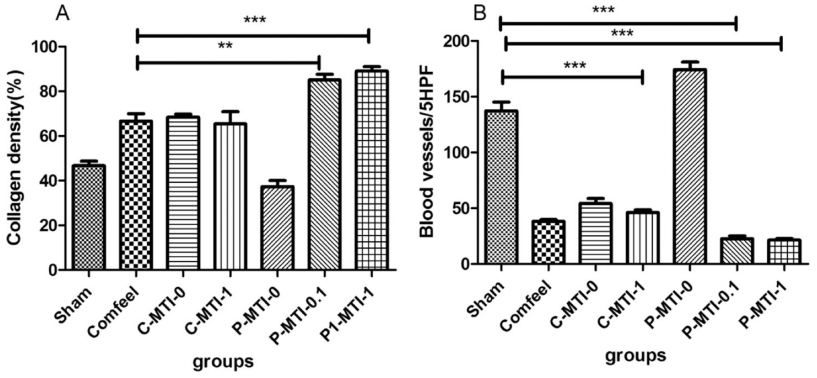


Figure 8. (A) Collagen density quantitative analysis and (B) angiogenesis quantitative analysis of C-MTI-0, C-MTI-1, P-MTI-1, P-MTI-0.1, P-MTI-0, sham negative control, and Comfeel positive control. * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

epithelialization compared to the negative control (***$P < 0.001$). Furthermore, the highest amount of efficacy was observed for the patch groups, the cream with Matrixyl groups, and Comfeel group (**$P < 0.01$).

5. CONCLUSIONS

This study indicated the importance of active substances on the wound healing process, as well as its mode of delivery, cream vs patch. Our analysis showed that the patch groups administered

https://doi.org/10.1021/acsomega.2c02592
ACS Omega 2022, 7, 24695−24704

Table 3. Histomorphometric Analysis of Different Experimental Groups

group	angiogenesis/SHPF (n = 3)	collagen content (%) (n = 3)
C-MTI-0	54.3 ± 7.7 (21 days)	68.3 ± 2.5 (21 days)
C-MTI-1	46 ± 4.5 (21 days)	65.3 ± 9.6 (21 days)
P-MTI-0	174.3 ± 11.6 (21 days)	37.3 ± 4.7 (21 days)
P-MTI-1	21.3 ± 2.5 (21 days)	89 ± 3.6 (21 days)
P-MTI-0.1	22.6 ± 4.5 (21 days)	85 ± 4.5 (21 days)
sham	137.3 ± 13.6 (21 days)	46.6 ± 3.5 (21 days)
Comfeel	38.3 ± 2.5 (21 days)	66.6 ± 5.6 (21 days)

Table 4. Histomorphometric Analysis of Different Experimental Groups[a]

group	epitheliogenesis score (N = 3)
C-MTI-0	2, 3, 2 (21 days)*
C-MTI-1	3, 3, 3 (21 days)**
P-MTI-0	2, 1, 1 (21 days)
P-MTI-1	3, 4, 3 (21 days)***
P-MTI-0.1	4, 3, 4 (21 days)***
sham	1, 1, 1 (21 days)
Comfeel	3, 3, 2 (21 days)**

[a]*, **, ***: values indicate treatment group vs nontreatment (sham) group; *P < 0.05, **P < 0.01, ***P < 0.001.

with active substance peptides in comparison to other treatment groups contributed to an improved wound healing process with the best cosmetic appearance, with normal thickness of the epidermal layer and the rejuvenation of the hair follicles and skin appendages. In contrast, the control negative group (sham), at all days of treatment, showed polymorphonuclear inflammatory cell (PMN) infiltration and granulation tissue formation. In fact, the epidermal layer could not be formed even by the end of day 21 and the wound was covered by a crusty scab. Micrographs of the patch without Matrixyl groups (P-MTI-0) showed similar results to the negative control group by forming a crusty scab covering the inflamed wound area without the epidermal formation. This indicates the necessity of the presence of MTI owing to its therapeutic effects in wound healing.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c02592.

Kiaser test protocol; quality control tests after Matrixyl synthesis including mass spectroscopy of synthesized Matrixyl (Figure S1); Matrixyl assay in both synthesized samples and purchased Matrixyl powder as standard (Figure S2 and Table S1); purity measurement of synthesized Matrixyl (Figure S3 and Table S2); residual solvent after Matrixyl synthesis (Figure S4); pH measurements of Matrixyl (Table S3); and moisture content of Matrixyl (Table S4) (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Mohammad Raoufi − *Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1416634793, Iran; Physical Chemistry I, Department of Chemistry and Biology & Research Center of Micro and Nanochemistry and Engineering (Cμ), University of Siegen, 57076 Siegen, Germany;* orcid.org/0000-0003-4176-7729; Email: mraoufib@gmail.com

Mohammad Sharifzadeh − *Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1416634793, Iran;* Email: msharifzadeh@tums.ac.ir

Authors

Maryam Kachooeian − *Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1416634793, Iran*

Zahra Mousivand − *Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1416634793, Iran*

Elham Sharifikolouei − *Department of Applied Science and Technology, Politecnico di Torino (POLITO), 10129 Turin, Italy*

Mehrnoosh Shirangi − *Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1416634793, Iran*

Loghman Firoozpour − *Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 1416634793, Iran*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c02592

Author Contributions

M.K. contributed to the conceptualization, methodology, and writing of the original draft. Z.M. contributed to patch synthesis. E.S. contributed to reviewing, editing, and validation. Mehrnoosh Shirangi contributed to test validation and discussion. L.F. contributed to the validation and discussion. M.R. contributed to the writing of the original draft, editing, reviewing, and validation. Mohammad Sharifzadeh contributed to in vivo tests.

Notes

The authors declare no competing financial interest.

In vitro and in vivo experiments were registered at the ethics committee of the Tehran University of Medical Sciences (TUMS), Iran, and confirmed by its ethics committee.

■ ACKNOWLEDGMENTS

The authors are thankful to the Tehran University of Medical Sciences for the funding and financial support of the project.

■ REFERENCES

(1) Kolarsick, P. A. J.; Kolarsick, M. A.; Goodwin, C. Anatomy and physiology of the skin. *J. Dermatol. Nurses' Assoc.* **2011**, *3*, 203−213.

(2) Gomes, A.; Teixeira, C.; Ferraz, R.; Prudêncio, C.; Gomes, P. Wound-healing peptides for treatment of chronic diabetic foot ulcers and other infected skin injuries. *Molecules* **2017**, *22*, 1743.

(3) Su, L.; Zheng, J.; Wang, Y.; Zhang, W.; Hu, D. Emerging progress on the mechanism and technology in wound repair. *Biomed. Pharmacother.* **2019**, *117*, No. 109191.

(4) Sonnemann, K. J.; Bement, W. M. Wound repair: toward understanding and integration of single-cell and multicellular wound responses. *Annu. Rev. Cell Dev. Biol.* **2011**, *27*, 237−263.

(5) (a) Fosgerau, K.; Hoffmann, T. Peptide therapeutics: current status and future directions. *Drug Discovery Today* **2015**, *20*, 122−128. (b) Uhlig, T.; Kyprianou, T.; Martinelli, F. G.; Oppici, C. A.; Heiligers, D.; Hills, D.; Calvo, X. R.; Verhaert, P. The emergence of peptides in the pharmaceutical business: From exploration to exploitation. *EuPA Open Proteomics* **2014**, *4*, 58−69. (c) Hemu, X.; El Sahili, A.; Hu, S.; Wong,

K.; Chen, Y.; Wong, Y. H.; Zhang, X.; Serra, A.; Goh, B. C.; Darwis, D. A.; et al. Structural determinants for peptide-bond formation by asparaginyl ligases. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 11737−11746.
(6) Chakrabarti, S.; Jahandideh, F.; Wu, J. Food-derived bioactive peptides on inflammation and oxidative stress. *BioMed Res. Int.* **2014**, *2014*, No. 608979.
(7) Schagen, S. K. Topical peptide treatments with effective anti-aging results. *Cosmetics* **2017**, *4*, 16.
(8) Abu Samah, N. H.; Heard, C. M. Topically applied KTTKS: a review. *Int. J. Cosmetic Sci.* **2011**, *33*, 483−490.
(9) (a) Robinson, L. R.; Fitzgerald, N.; Doughty, D.; Dawes, N.; Berge, C.; Bissett, D. Topical palmitoyl pentapeptide provides improvement in photoaged human facial skin 1. *Int. J. Cosmetic Sci.* **2005**, *27*, 155−160. (b) Choi, Y. L.; Park, E. J.; Kim, E.; Na, D. H.; Shin, Y.-H. Dermal stability and in vitro skin permeation of collagen pentapeptides (KTTKS and palmitoyl-KTTKS). *Biomol. Ther.* **2014**, *22*, 321.
(10) Aldag, C.; Teixeira, D. N.; Leventhal, P. S. Skin rejuvenation using cosmetic products containing growth factors, cytokines, and matrikines: a review of the literature. *Clin. Cosmetic Invest. Dermatol.* **2016**, *9*, 411.
(11) Jones, R. R.; Castelletto, V.; Connon, C. J.; Hamley, I. W. Collagen stimulating effect of peptide amphiphile C16−KTTKS on human fibroblasts. *Mol. Pharmaceutics* **2013**, *10*, 1063−1069.
(12) Lintner, K.; Mas-Chamberlin, C.; Mondon, P.; Peschard, O.; Lamy, L. Cosmeceuticals and active ingredients. *Clin. Dermatol.* **2009**, *27*, 461−468.
(13) Park, H.; An, E.; Lee, A.-R. C. Effect of palmitoyl-pentapeptide (Pal-KTTKS) on wound contractile process in relation with connective tissue growth factor and α-smooth muscle actin expression. *Tissue Eng. Regener. Med.* **2017**, *14*, 73−80.
(14) Mahdavi, H.; Kermani, Z.; Faghihi, G.; Asilian, A.; Hamishehkar, H.; Jamshidi, A. Preparation and evaluation of cosmetic patches containing lactic and glycolic acids. *Indian J. Dermatol. Venereol. Leprol.* **2006**, *72*, 432−436.
(15) Prabhakar, D.; Sreekanth, J.; Jayaveera, K. Transdermal drug delivery patches: a review. *J. Drug Delivery Ther.* **2013**, *3*, 231.
(16) Dhiman, S.; Singh, T. G.; Rehni, A. K. Transdermal patches: a recent approach to new drug delivery system. *Int. J. Pharm. Pharm. Sci.* **2011**, *3*, 26−34.
(17) Al Musaimi, O.; Beatriz, G.; Albericio, F. Greening Fmoc/t Bu solid-phase peptide synthesis. *Green Chem.* **2020**, *22*, 996−1018.
(18) (a) Atashgah, R. B.; Ghasemi, A.; Raoufi, M.; Abdollahifar, M.-A.; Zanganeh, S.; Nejadnik, H.; Abdollahi, A.; Sharifi, S.; Lea, B.; Cuerva, M.; et al. Restoring Endogenous Repair Mechanisms to Heal Chronic Wounds with a Multifunctional Wound Dressing. *Mol. Pharmaceutics* **2021**, *18*, 3171−3180. (b) Raoufi, M.; Das, T.; Schoen, I.; Vogel, V.; Brüggemann, D.; Spatz, J. P. Nanopore diameters tune strain in extruded fibronectin fibers. *Nano Lett.* **2015**, *15*, 6357−6364. (c) Raoufi, M.; Aslankoohi, N.; Mollenhauer, C.; Boehm, H.; Spatz, J. P.; Brüggemann, D. Template-assisted extrusion of biopolymer nanofibers under physiological conditions. *Integr. Biol.* **2016**, *8*, 1059−1066.
(19) DiPietro, L. A. Angiogenesis and wound repair: when enough is enough. *J. Leukocyte Biol.* **2016**, *100*, 979−984.
(20) Katayama, K.; Armendariz-Borunda, J.; Raghow, R.; Kang, A.; Seyer, J. A pentapeptide from type I procollagen promotes extracellular matrix production. *J. Biol. Chem.* **1993**, *268*, 9941−9944.

https://doi.org/10.1021/acsomega.2c02592
ACS Omega 2022, 7, 24695−24704