ARHLFW: More than a cosmetic peptide

Y. Sarigiannis[1], A. Miliotou[2], T. Panayi[2], Ch. Petrou[2], P. Rodosthenous[2], P. Kaloumenos[2], C.M. Samiotou[2], M.V. Fronimou[3], C.M. Mikelis[3]

[1] Department of Health Sciences, University of Nicosia, UNIC Athens, GR-16777 Athens, Greece
[2] Department of Health Sciences, University of Nicosia, CY-2417 Nicosia, Cyprus
[3] Department of Pharmacy, University of Patras, GR-26504 Patras, Greece
Contact: sarigiannis.i@unic.ac.cy

https://doi.org/10.17952/38EPS.2026.P193

Introduction

Ultra-short synthetic peptides are emerging as multifunctional bioactives for skin applications. The ARHLFW motif is known from its N-acetylated cosmetic analogue (Ac-ARHLFW-NH_2; marketed as Acetyl Hexapeptide-1), while the broader properties of the free-N-terminus H-ARHLFW-NH_2 remain poorly explored. Here, we investigate its physicochemical stability, cytocompatibility, wound-healing-associated gene modulation and antibacterial activity.

Peptide synthesis and Physicochemical Stability

An ultra fast method developed in-house applying MW-SPPS and employing green solvents was used for the synthesis of the peptide, reducing total synthesis time and solvent consumption in comparison with the conventional methods [1]. Identity and purity was confirmed by HPLC and LC-MS. A single dominant HPLC peak (R_t ≈ 13.3 min) and the intact LC-MS ion (m/z 828.7 $[M+H]^+$; 414.9 $[M+2H]^{2+}$) remain detectable from Day 1 to Day 6 in water and PBS — no new degradation peaks.

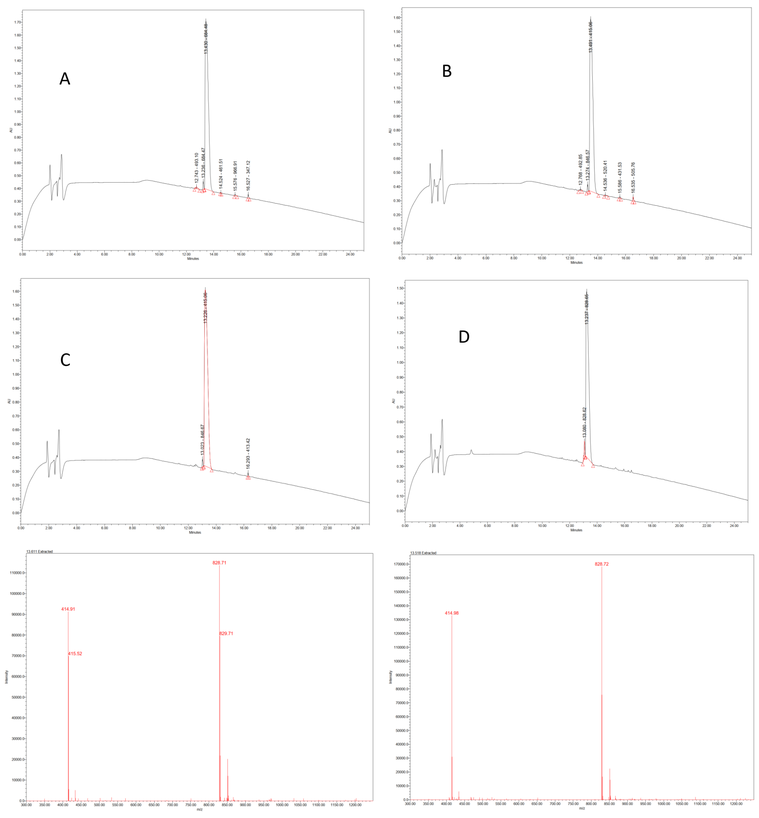


Fig 1. HPLC (220 nm) chromatograms and mass spectra of H-ARHLFW-NH_2 in water (A, C) and PBS (B, D) at Day 1 (up) and Day 6 (down). Day-6 chromatograms retain a dominant peak at approximately 13.0–13.2 min, while peptide-related MS signals remain detectable. HPLC Conditions: XBridge Peptide BEH C18 (4.6 × 150 mm, 2.5 µm, 300 Å); 0.1% formic acid in H_2O/ACN, 5–65% B over 25 min, 0.8 mL min^{-1}, 5 µL injection, detection at 220/280 nm. MS was used for molecular-ion confirmation.

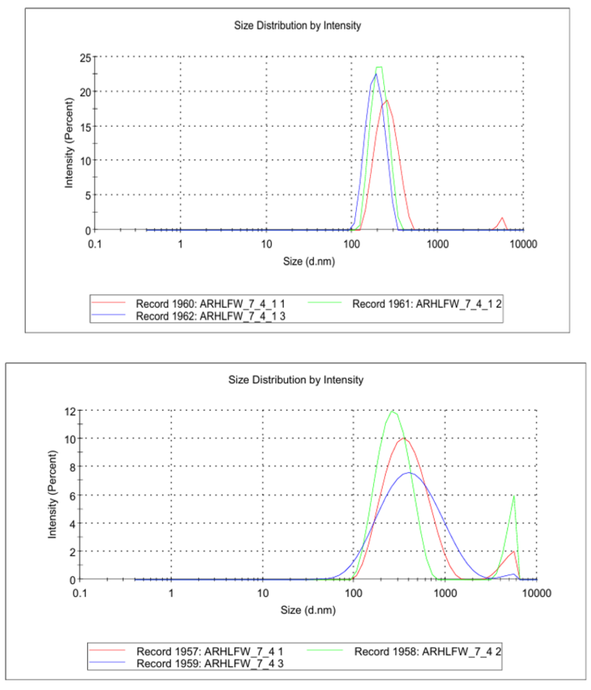


Fig 2. Dynamic Light Scattering (DLS) of ARHLFW at 25°C in Water (up) and PBS pH 7.4 (down). At 25°C, ARHLFW forms larger and more heterogeneous assemblies in PBS than in water (324.7 vs 260.5 nm; PDI 0.338 vs 0.285), highlighting the influence of ionic environment on self-assembly.

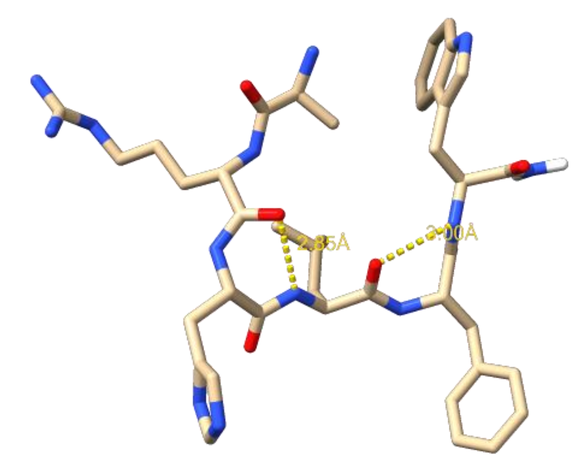


Fig 3. AlphaFold 3-predicted conformation of H-ARHLFW-NH_2, visualised in ChimeraX 1.11.1 after post-prediction C-terminal amidation. Short intramolecular backbone O···N contacts (2.85 and 3.00 Å) are consistent with a compact turn-like arrangement.

Biocompatibility — HaCaT & MDA-MB-231 cells

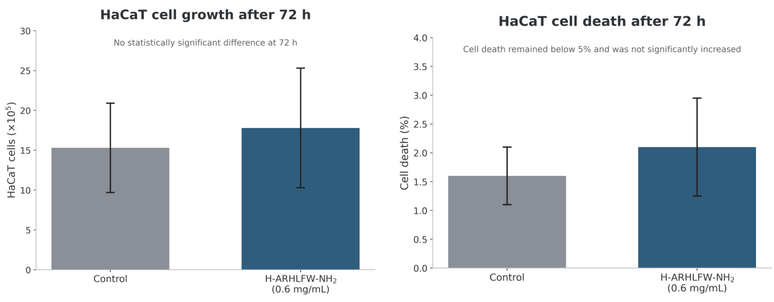


Fig 4. HaCaT response to H-ARHLFW-NH_2 (0.6 mg/mL, 72 h): cell growth was maintained and cell death remained low, with no statistically significant difference versus control (n=3).

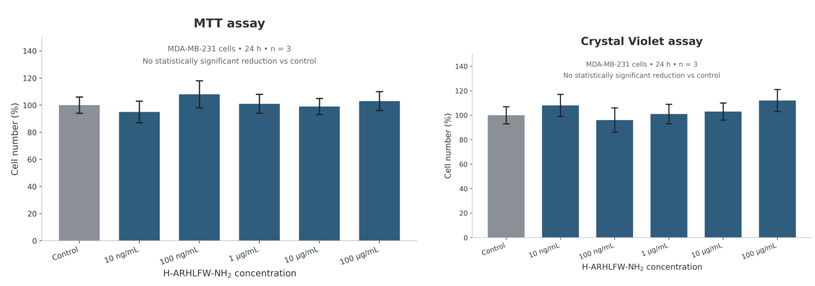


Fig 5. MDA-MB-231 cells exposed to H-ARHLFW-NH_2 for 24 h showed no statistically significant reduction in proliferation or survival across 10 ng/mL–100 µg/mL by either MTT or Crystal Violet assays (n=3).

Wound-healing associated transcriptional activity

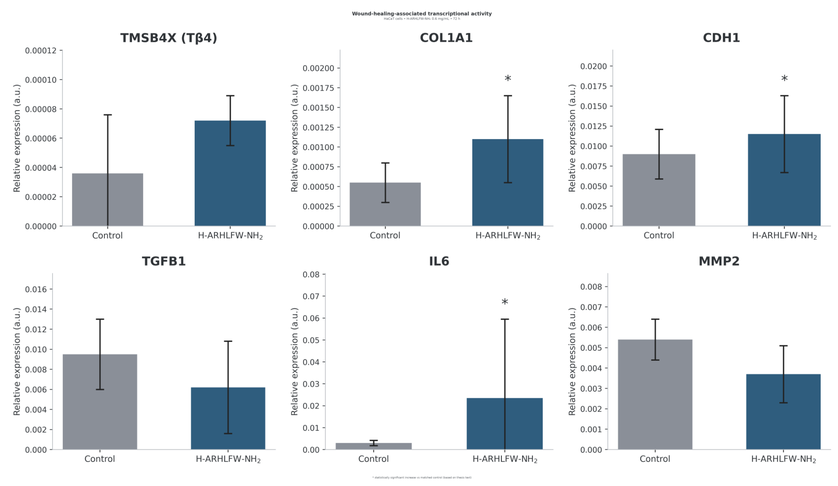


Fig 6. H-ARHLFW-NH_2 induced a distinct repair-associated transcriptional profile in HaCaT cells, characterised by increases in COL1A1, IL6 and CDH1, together with an upward trend in TMSB4X expression, without parallel upregulation of TGFB1 or MMP2 (n=3) (*P<0.05).

Proposed wound-healing-associated transcriptional programme

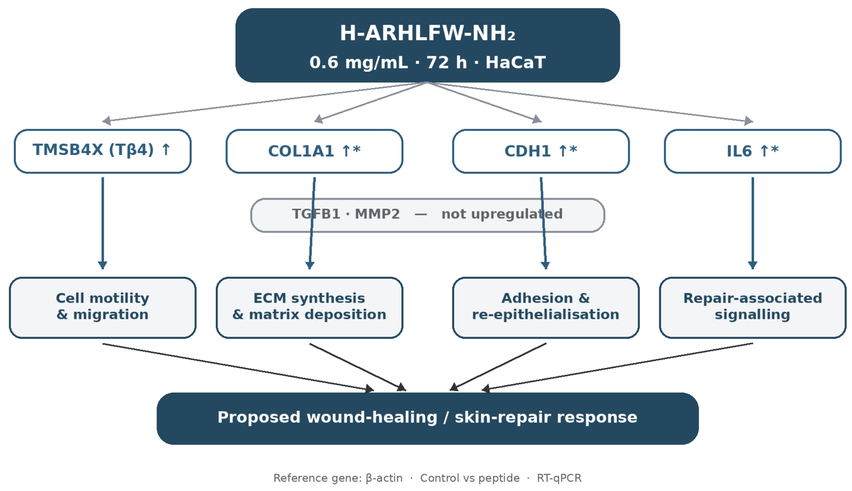


Fig 7. Schematic interpretation of the H-ARHLFW-NH_2-associated transcriptional response, integrating ECM support, epithelial cohesion and repair-related signaling.

Antimicrobial activity

H-ARHLFW-NH_2 was evaluated at 2% (w/v) against *E. coli* (ATCC-700926) , *S. Epidermidis* (ATCC-14990) and *N. Subflava* (ATCC-49275) using solid-lawn and 24 h liquid-culture assays. Ampicillin and WFI were used as positive and negative controls, respectively.

Liquid cultures: 4-log_{10} CFU/mL reduction was observed for both *E. Coli* and *S. Epidermidis* (~99.99% reduction), whereas *N. Subflava* showed little or no response.

Solid bacterial lawns: Visible growth inhibition was observed against all three tested species, indicating that the apparent activity of H-ARHLFW-NH_2 depends on the assay environment.

H-ARHLFW-NH_2 displayed marked antibacterial activity against *E. coli* and *S. epidermidis* in liquid culture, while the solid/lawn assays revealed broader but assay-dependent inhibition.

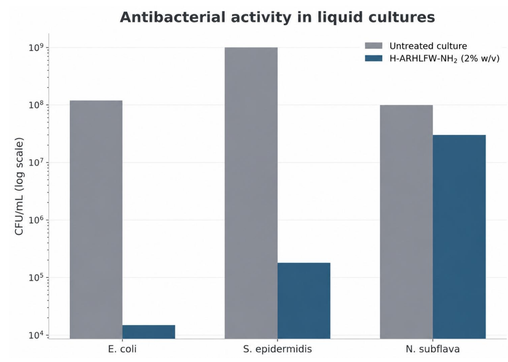


Fig 8. H-ARHLFW-NH_2 (2% w/v) produced an approximately 4-log_{10} reduction in viable E. coli and S. epidermidis after 24 h, while N. subflava showed little response.

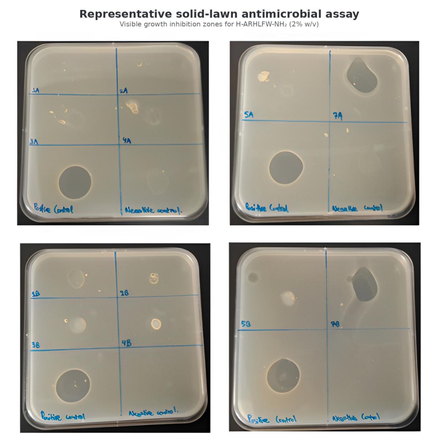


Fig 9. Solid-lawn assays, H-ARHLFW-NH_2 (2% w/v) produced visible growth-inhibition zones against all three tested bacterial species, indicating broader but assay-dependent antibacterial activity.

Take Home Message: H-ARHLFW-NH_2 emerges as more than a cosmetic peptide, combining physicochemical stability, cellular compatibility, repair-associated transcriptional activity and antibacterial effects that support its further investigation for skin-repair and topical applications.

References

[1]. Topouzidou, N et al., Unraveling the Molecular Mechanisms of Synthetic Acetyl Hexapeptide in E-Cadherin Activation for Tissue Rejuvenation. *Cosmetics* **2025**, *12*, 48. https://doi.org/10.3390/cosmetics12020048.