WAAPV-loaded electrospun PCL/PEG films for inhibiting elastase and bacteria activities in diabetic ulcer-like scenarios

Ana R. M. Ribeiro[1,#], Catarina S. Miranda[1,#], Filipa D.P. Mendes[2], Sílvia M. M. A. Pereira-Lima[2], Susana P. G. Costa[2], and Helena P. Felgueiras[1,*]

*[1]Centre for Textile Science and Technology (2C2T), University of Minho, Campus of Azurém, 4800-058 Guimarães, Portugal: anarribeiro@2c2t.uminho.pt (A.R.M.R.); catarina.miranda@2c2t.uminho.pt (C.S.M.); helena.felgueiras@2c2t.uminho.pt (H.P.F.). [#] Co-first authors; * Corresponding author.*

[2]Centre of Chemistry (CQ), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal: filipa.mendes131@gmail.com (F.D.P.M.); silviap@quimica.uminho.pt (S.M.M.A.P.L.); spc@quimica.uminho.pt (S.P.G.C.)

Abstract: Diabetic ulcers, like any other chronic wound, pose formidable clinical challenges, marked by the wounds' inability to progress through the usual stages of healing and becoming trapped in the inflammatory phase. As inflammation persists, neutrophils are recruited to the wound site, leading to increased secretion of human neutrophil elastase (HNE). The disproportionate presence and activity of HNE in diabetic ulcers have profound implications for the healing process. HNE's enzymatic activity targets various components critical for wound repair, including the ECM proteins and growth factors, both endogenous and supplemental [1]. In efforts to counteract the harmful effects of HNE in diabetic ulcers, researchers have explored the tetrapeptide Alanine-Alanine-Proline-Valine (AAPV). AAPV acts as an antagonist to HNE, presenting a promising strategy for regulating its function. The hydrophobic sequences inherent in AAPV share structural similarities with amino acid regions present in elastin, a key component of the ECM. Leveraging these structural resemblances, AAPV binds competitively to the active sites of HNE, the sub-sites P-P1, effectively impeding its enzymatic activity. By limiting HNE's actions, AAPV contributes to preserving the ECM's structural integrity and safeguarding crucial growth factors essential for the ulcer healing process [2].

Despite its efficacy, the widespread pharmaceutical application of peptides faces obstacles, including limited stability in physiological settings and susceptibility to environmental factors. Also, AAPV's overall hydrophilic nature (despite the presence of hydrophobic domains) tends to reduce its bioavailability and cellular tissue permeability. The attachment of hydrophobic carriers/molecules can overcome these limitations. Tryptophan (W) is an aromatic hydrophobic amino acid which incorporation into peptide sequences has been associated with the activation of antibacterial properties (disturbance of bacterial membrane). Considering the abnormal response of HNE is aggravated by the presence of bacteria pathogens in the ulcer bed, the addition of antibacterial amino acids to therapeutic peptides may offer supplemental opportunities for promoting healing. Attending to this information, it was postulated the incorporation of tryptophan at the N-terminal of the peptide, generating WAAPV, for improving the AAPV's permeability and antibacterial capacity while maintaining its activity against HNE.

For effective topical delivery, an electrospun fibrous film made of polycaprolactone (PCL)/polyethylene glycol (PEG), loaded with the formulated peptide sequences, was developed and used as a carrier. Through this structure, a topical release of AAPV and WAAPV peptides was ensured for effective treatment of diabetic ulcers. Data reported the successful production of a porous film structure, made of continuous, uniformly distributed fibers. Their stability in physiological medium was also verified up to 28 days of incubation. Wettability was increased in the presence of the PEG polymer and the peptides. Furthermore, it was demonstrated the inhibitory potential of WAAPV against HNE (above the AAPV tetrapeptide) and bacteria (co-adjuvant effects observed between the peptides and PEG). Finally, through these films the first steps towards establishing WAAPV as a candidate/model for new multi-action therapies in diabetic ulcers' care were taken.

Keywords: *small peptides; electrospinning; wound healing; inhibitory action.*

ACKNOWLEDGEMENT*: This work was funded by the Portuguese Foundation for Science and Technology of Portugal (FCT) under the project UID/CTM/00264/2020 of 2C2T. Additionally, authors acknowledge funding received by the Portuguese Society of Diabetology under the Luis Marques SPD scholarships. A.R.M.R. and C.S.M. acknowledge FCT for PhD funding via scholarships 2021.08627.BD and 2020.08547.BD, respectively, and H.P.F. for auxiliary researcher contract https://doi.org/10.54499/DL57/2016/CP1377/CT0098.*

REFERENCES

[1] Felgueiras, HP, Emerging antimicrobial and immunomodulatory fiber-based scaffolding systems for treating diabetic foot ulcers. Pharmaceutics, 2023;15(1):258.

[2] Miranda, CS, *et al.* Sodium alginate/ polycaprolactone co-axial wet-spun microfibers modified with N-carboxymethyl chitosan and the peptide AAPV for Staphylococcus aureus and human neutrophil elastase inhibition in potential chronic wound scenarios. Biomaterials Advances, 2023;151:213488.