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Novel approach for the High-yield expression and Purification of Bio- Active LL-37: Implications for Biomedical Research

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Abstract

LL-37, the sole human cathelicidin, is a multifunctional antimicrobial peptide with potential applications in wound healing due to its chemotactic, endotoxin-neutralizing, and angiogenic properties. Previous recombinant production approaches have typically involved the addition of N-terminal fusion proteins to enhance peptide expression, requiring complex purification processes that diminish yield and increase costs. This study presents the first successful recombinant production of LL-37 in its active form without N-terminal fusions. The LL-37 gene sequence was cloned with a G4S linker and hydroxyapatite binding domain into the pET21a(+) vector and expressed in *E. coli* Shuffle. The recombinant peptide was purified in a single step via affinity chromatography, achieving a yield of 1.02 mg of LL-37 per liter of culture. To evaluate any effects of C-terminal fused sequences on LL-37 activity, a series of assays were conducted. Antimicrobial assays demonstrated reduced activity of the recombinant LL-37 against *E. coli* and *S. aureus* compared to its native counterpart. Enzyme-linked immunosorbent assay (ELISA) revealed that the recombinant peptide binds to lipopolysaccharides (LPS) in a dose-dependent manner ($p < 0.05$), confirming its endotoxin-neutralizing capabilities. Wound healing potential was assessed using cell scratch assays on human umbilical vein endothelial cells (HuVEC) and human dermal fibroblasts (HDF), showing that recombinant LL-37 (100 ng/mL) significantly enhanced cell migration, achieving an 86% wound repair rate in contrast to 14% in controls after 12 hours ($p < 0.05$). Additionally, a cell-based ELISA confirmed the binding of recombinant LL-37 to endothelial cell surface receptors in a dose-dependent manner. Importantly, the recombinant LL-37 displayed no cytotoxic effects on HuVEC and fibroblast cells, even at concentrations up to 25 µg/mL. These findings suggest that the recombinant LL-37 can be produced efficiently without

fusion tags, retaining its biological activities, and have significant implications for its use in therapeutic applications for wound healing.

Key words: LL-37 peptide, antimicrobial peptide, wound healing, cost benefit expression and purification.