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Development of a cell-based platform for expression of antimicrobial peptides: modification of HEK-293T cells for expression and extracellular secretion of human β-defensin 1

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INTRODUCTION & AIM

Antibiotic resistance is recognized as a major public health threat. Antimicrobial peptides provide an alternative to antibiotics. Human β-defensin 1 (hBD1), produced by epithelial cells, demonstrates broad antimicrobial activity and retains stability after proteolysis. However, its clinical application is constrained by high synthesis costs.

This study aimed to generate a eukaryotic cell platform for hBD1 expression and secretion.

METHOD

Plasmids pHBD1-Out and pHBD1RFP-Out were designed using the pcDNA3.1(+) vector with the CMV promoter. Cloning was performed in *E. coli* XL1-Blue, followed by plasmid purification and restriction analysis. HEK-293T cells were transfected via lipofection. Transfection efficiency was evaluated after 48 h by fluorescence microscopy. hBD1 production was confirmed by PAGE and HPLC. Antimicrobial activity was assessed against E. coli using agar diffusion assay.

RESULTS & DISCUSSION

The pHBD1-Out plasmid carried the hBD1 sequence and the SRP9 signal peptide for extracellular expression, while pHBD1RFP-Out additionally contained A2P peptide and RFP marker (Fig. 1).

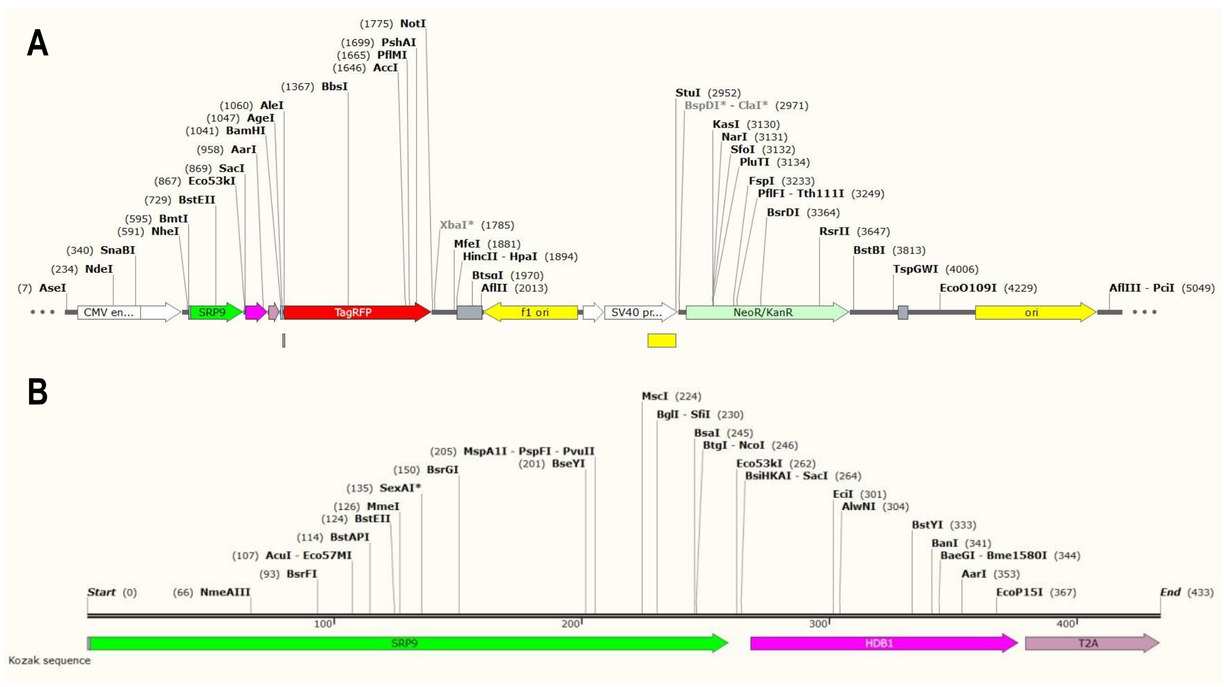

Figure 1. Plasmid maps of pHDB1-Out *(5107 bp)* (**A**) and gene insertion vHDB1-Out *(433 bp)* (**B**).

Transfection was confirmed by the presence of fluorescent cells, although the efficiency was relatively low (Fig. 2, 3).

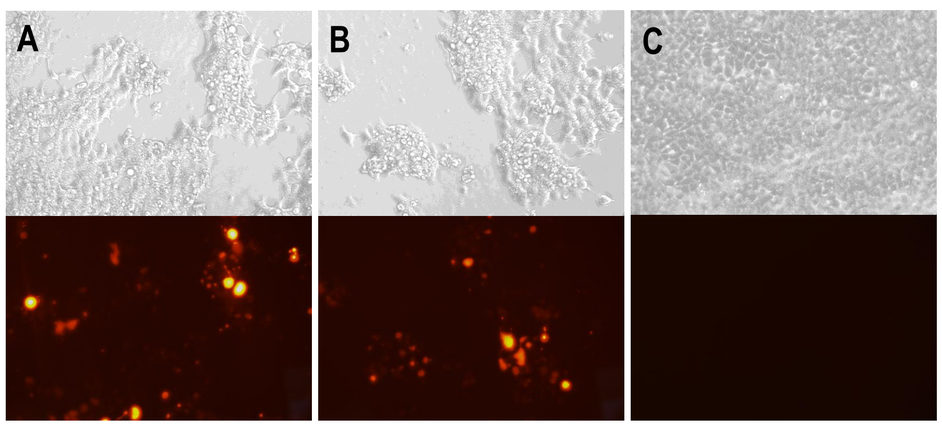

Figure 2. HEK-293T cells transfected with pRFP (**A**), pHBDRFP-Out (**Б**) and pHBD-Out (**B**) using 2000 ng of plasmids and GenJect-39. 48 h after transfection.

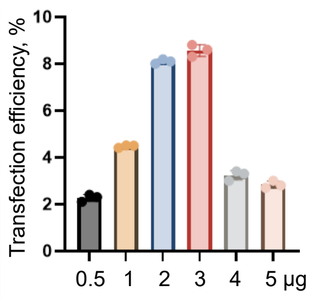

Figure 3. Transfection efficiency of HEK-293T cells depending from plasmid DNA concentration.

hBD1 production was detected in both cell lysates and supernatants, showing similar expression patterns for pHBD1-Out and pHBD1RFP-Out (Fig. 4).

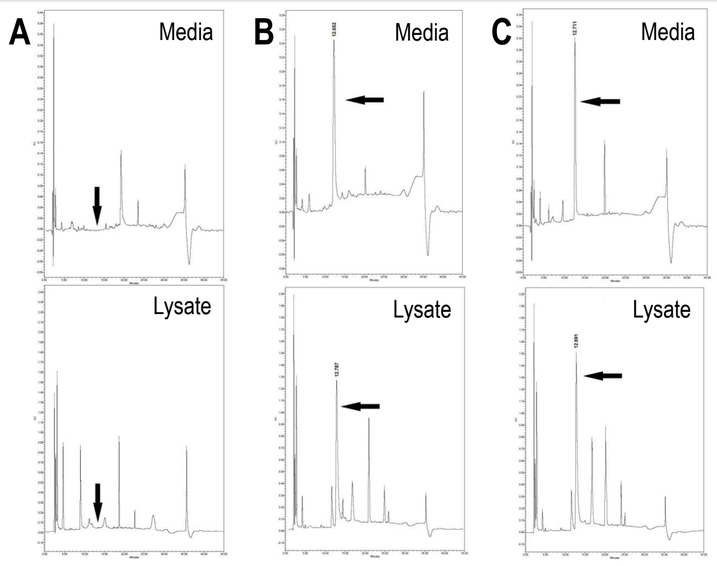

Figure 4. HPLC analysis of lysates and supernatants from HEK-293T cells transfected with pRFP (**A**), pHBDRFP-Out (**B**) and pHBD-Out (**C**). Arrow indicates the hBD1 peak. The average retention time was 12.7 min.

Lysates demonstrated pronounced antimicrobial activity comparable to ampicillin (Fig. 5). Supernatants also inhibited *E. coli* growth, though less effectively.

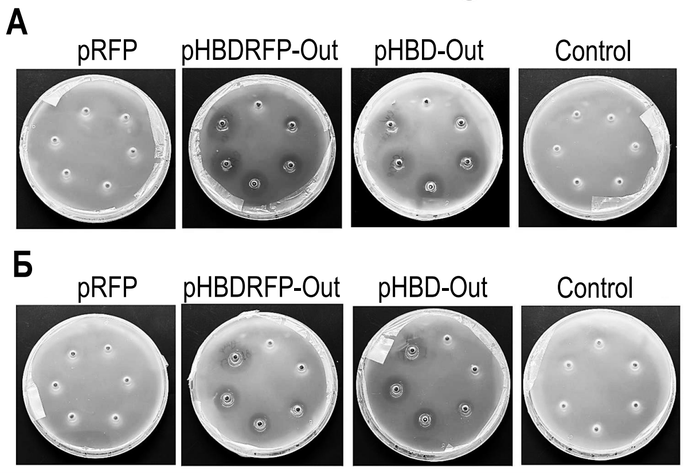

Figure 5. Antimicrobial activity test of lysates (**A**) and supernatants (**B**) from transfected HEK-293T cells against *E. coli*. Intact HEK-293T cell were used as “Control”.

CONCLUSION

The constructed plasmids enabled efficient hBD1 expression in HEK-293T cells. The recombinant peptide exhibited antimicrobial activity comparable to conventional antibiotics, highlighting the potential of cell-based systems for AMP production and the development of novel antimicrobial therapeutics.

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