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DEVELOPMENT OF A SELF-HEALING BIOMATERIAL BASED ON MODIFIED RECOMBINANT FIBROIN FOR BIOMEDICAL APPLICATION

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Abstract

Self-healing biomaterials are relevant for regenerative medicine due to their ability to restore mechanical integrity after damage. Spider dragline silk fibroin is a suitable platform because of its high strength, elasticity, and biocompatibility. In this study, recombinant fibroin was modified to enhance potential self-healing properties through incorporation of hydrophobic and tyrosine-containing motifs associated with reversible supramolecular interactions. Hybrid constructs were designed in silico and structurally evaluated prior to experimental implementation. A 1× fibroin gene was constructed, codon-optimized for *E. coli*, and cloned into a T7-based vector, followed by in-plasmid duplication to generate a 4× repeat variant. To overcome translational limitations typical for repetitive silk genes, a SUMO fusion strategy with an RFP reporter was applied. The SUMO-1×-RFP (~43 kDa) and SUMO-4×-RFP (~54 kDa) proteins were successfully expressed in *E. coli* after IPTG induction, as confirmed by SDS-PAGE and fluorescence analysis. The obtained results establish a platform for further production of higher-order constructs and subsequent mechanical and self-healing characterization.

Keywords
Recombinant fibroin, spider silk, self-healing biomaterials, medical biomaterials

Introduction: Self-healing biomaterials represent a promising direction in regenerative medicine, where mechanical durability and long-term stability are critical. Materials capable of restoring their structural integrity after damage can significantly improve the lifespan of implants and tissue scaffolds. Spider dragline silk fibroin is an attractive candidate due to its exceptional tensile strength, elasticity, and biocompatibility [1].

Recent studies suggest that self-healing in silk-based materials is mediated by reversible supramolecular interactions, including β-sheet reorganization, hydrogen bonding, and aromatic stacking. We hypothesized that targeted modification of fibroin sequences by introducing hydrophobic and tyrosine-containing motifs into β-sheet and amorphous domains would enhance self-healing performance while preserving mechanical robustness [2,3].

Main part: Hybrid fibroin constructs were first designed in silico using sequence-based predictive models to estimate mechanical parameters including strain at break and Young's modulus [3]. Structural organization was evaluated with AlphaFold modeling to ensure preservation of β-sheet domains and elastic regions. Based on computational assessment, modified sequences containing GPGGX motifs with aromatic residues were selected.

The experimental work began with construction of a 1× recombinant fibroin gene, codon-optimized for *E. coli* and cloned into a T7 promoter-based expression vector. After validation, the 1× unit was duplicated within the vector to generate a 4× repeat construct.

Previous attempts to express repetitive constructs demonstrated transcription without detectable protein production, suggesting translational limitations typical for highly repetitive silk genes. To address this issue, a SUMO fusion strategy was implemented. A SUMO domain was fused to the 5′ end of the fibroin gene followed by an RFP reporter to directly monitor translation efficiency.

The resulting SUMO-1×-RFP fusion protein (~43 kDa) and its duplicated version

SUMO-4×-RFP (~54 kDa) were successfully expressed in *E. coli* BL21 and Rosetta strains after IPTG induction. SDS-PAGE analysis revealed a clear band corresponding to the expected molecular weight, and fluorescence of induced cultures confirmed active translation. These results demonstrate that SUMO fusion significantly improves expression stability and provides a reliable platform for further production of repetitive fibroin constructs.

Conclusion: In this work, a recombinant fibroin construct was developed. A 1× gene was first obtained and then duplicated in the plasmid to generate a validated 4× repeat variant. SUMO fusion enabled successful expression of the SUMO-4×-RFP protein (~54 kDa), confirming improved translation efficiency of repetitive silk sequences. Further work is focused on obtaining an 8× repeat construct and subsequent production of the corresponding protein for mechanical and self-healing characterization.

Literature

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