with Elastin-Like Polypeptide Fusion for Enhanced Tissue Regeneration

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Abstract: The study aimed to address the challenge posed by the large molecular weight of natural extracellular matrix (ECM) proteins in fabricating functional structures suitable for tissue repair. To overcome this, a low-molecular-weight and multifunctional chimeric recombinant ECM was engineered by fusing elastin-like polypeptide with various proteins to effectively stimulate mesenchymal stem cells (MSCs) for tissue regeneration. The rationale for establishing the fusion with elastin-like polypeptide was to enhance the bioactivity and functionality of the ECM proteins. Control studies with the proteins alone were conducted to assess the impact of elastin-like polypeptide fusion on cellular responses.

Additionally, the bio-functionalization of titanium surfaces with recombinant fibronectin and elastin-like peptide was utilized to enhance bioactivity for improved osseointegration. This biofunctionalization sustained bioactivity over a 4-week period without an initial burst effect and notably increased the adhesion, proliferation, and osteogenic differentiation of human mesenchymal stem cells (hMSCs). The biomimetic fibronectin-coated titanium surfaces further induced the elevated expression of osteogenesis-related genes, emphasizing its potential to promote bone regeneration.

Control studies with individual proteins and without elastin-like peptide fusion were conducted to evaluate the specific contribution of the fusion strategy to cellular responses. The results demonstrated significantly increased cellular activities and osteogenic differentiation on the biomimetic fibronectin-coated titanium compared to non-coated surfaces, highlighting the beneficial effects of elastin-like polypeptide fusion for enhancing tissue regeneration outcomes. In summary, the rationale for fusing elastin-like polypeptide to ECM proteins in this study is to leverage ELP's unique properties to enhance the biomimicry, solubility, stability, purification efficiency, controlled release, and overall bioactivity of recombinant ECM proteins for improved tissue regeneration applications. The fusion strategy offers a promising approach to overcome challenges associated with large-molecular-weight ECM proteins and optimize their therapeutic potential.

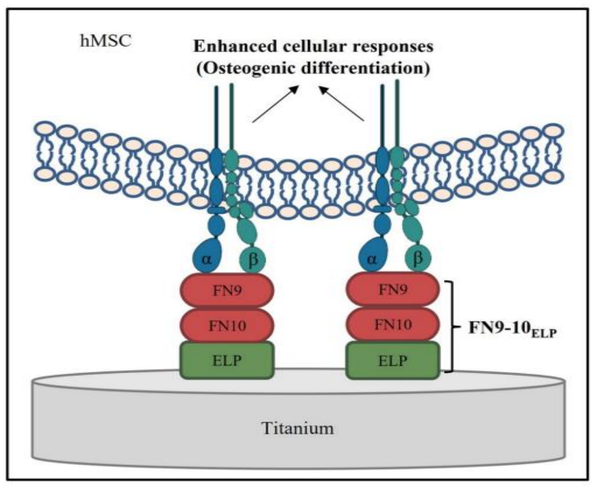
Introduction:

Implantable materials are essential in dental and orthopedic procedures . Titanium is widely used in the biomedical field due to its excellent biocompatibility and mechanical properties; however, it lacks inherent bioactivity for bone regeneration. Therefore, a key strategy to promote osteogenic stimuli involves enhancing cellular responses through the biofunctionalization of titanium surfaces with biomolecules. In this context, the extracellular matrix (ECM) acts as a biomolecule that provides a microenvironment to induce specific signaling pathways in stem cells, leading to positive effects [5,6]. Nonetheless, native ECM proteins have drawbacks such as immunological rejection and high production costs. Thus, an alternative approach involves utilizing biomimetic ECMs and biofunctionalizing titanium surfaces with recombinant ECM proteins.

Fibronectin (FN) is a vital constituent of the extracellular matrix (ECM) that plays a crucial role in regulating cellular functions including cell migration, proliferation, differentiation, and viability. The interaction between FN and integrin facilitates cell binding, particularly during osteoblast differentiation for bone formation, as evidenced by the results. Structurally, FN is a protein dimer composed of two closely similar monomers connected by a pair of C-terminal disulfide bonds. Each FN subunit contains homologous repeats of three types of modules (FNI, FNII, FNIII)

Results:

Fig. 1. Expression of chimeric FN9-10ELP and schematic illustration of bio-functionalization for enhanced cellular responses on titanium discs. (A) The purity and molecular weight of chimeric FN9-10ELP were measured by 12% SDS-PAGE and Western blotting. The molecular weight shown is approximately 38 kDa. (B) Bio-functionalization of titanium discs using chimeric FN9-10ELP to induce osteogenic differentiation of hMSCs.



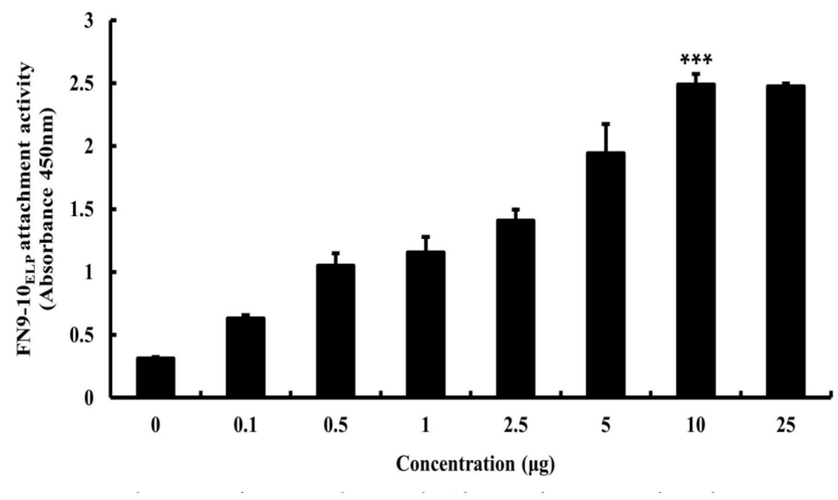


Fig.2. The protein attachment activity of FN9-10ELP on titanium discs was assessed. Titanium discs were immersed overnight in varying concentrations of FN9-10ELP (0−25 μg) in 6-well plates at 4˚C. The absorbance of FN9-10ELP was quantified using ELISA with a His-tag probe, and the results are reported as mean ± SD (n = 3), with a significance level of $p < 0.001$.

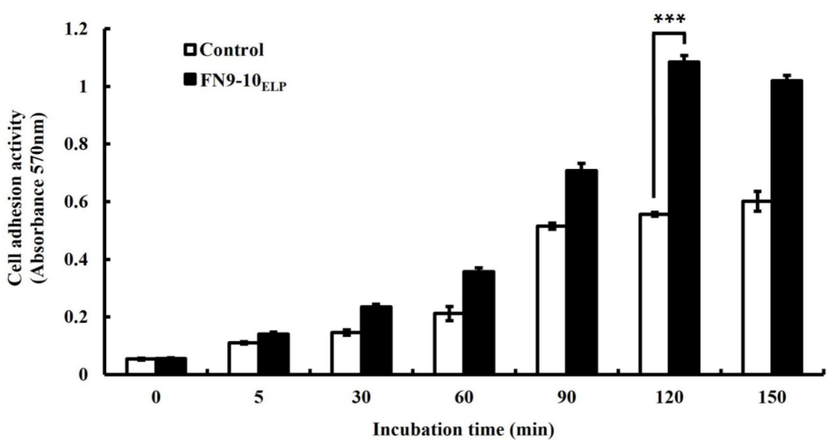


Fig. 3. Cell proliferation activity of human mesenchymal stem cells (hMSCs) on FN9-10ELP-coated titanium discs was assessed over 0, 4, and 8 days. Titanium discs were immersed in either 0 or 10 μg/mL FN9-10ELP overnight at 4˚C. Subsequently, hMSCs were seeded at a density of 1 × 10^4 cells/disc on the titanium discs and incubated for 0, 4, and 8 days at 37˚C. The absorbance of formazan produced by the cells was used as an indicator of cell proliferation. Cell proliferation activities are presented as mean ± SD (n = 3), with a significance level of $p < 0.001$.

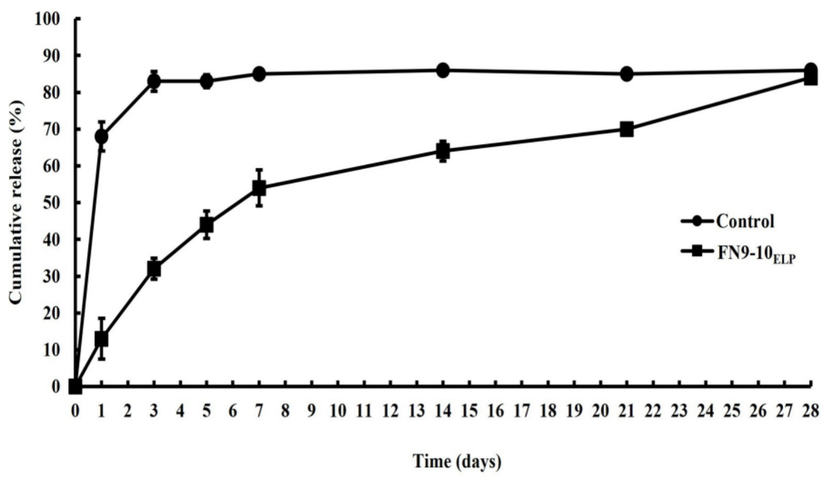


Fig. 4. The in vitro cumulative release profiles of FN9-10ELP from titanium discs were investigated. Titanium discs were coated with FN9-10ELP (10 μg/mL) and a control substance (5% BSA) in a volume of 500 μL/disc overnight at 4˚C. The amount of FN9-10ELP absorbed onto the titanium discs was quantified using an ELISA assay. The cumulative release was assessed relative to the initial attachment amount (100%). The release profiles are presented as mean ± SD (n = 3).

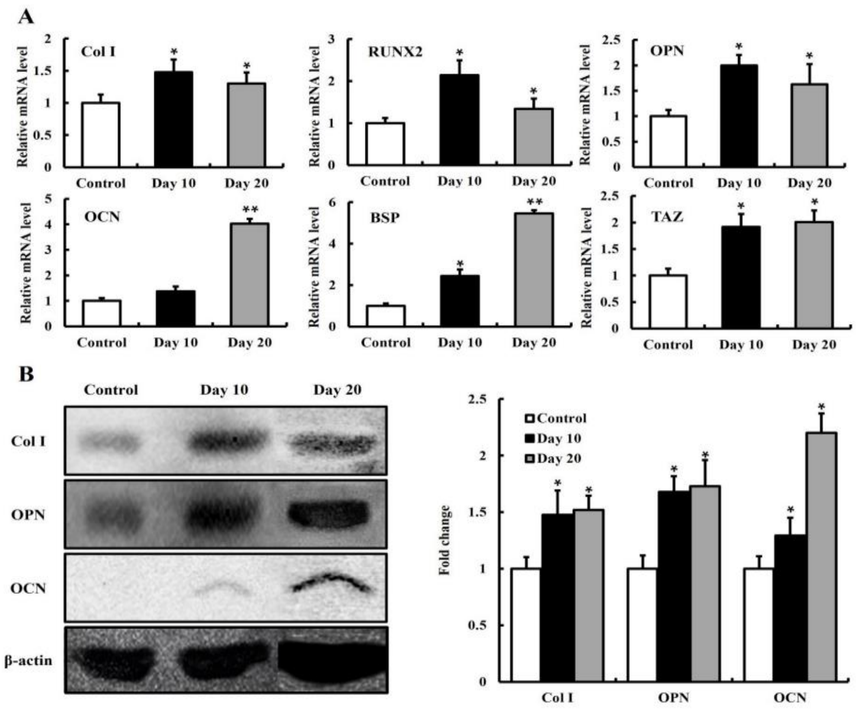


Fig. 5. Osteogenic differentiation activities of human mesenchymal stem cells (hMSCs) on FN9-10ELP-coated titanium discs were evaluated over a 20-day period. Titanium discs were coated by immersing them overnight in 10 μg/mL of FN9-10ELP at 4˚C, while non-coated discs served as controls. The hMSCs were seeded at a density of 5 × 10^3 cells/disc and incubated for 20 days at 37˚C.

(A) mRNA levels of osteogenesis-related genes (Col I, RUNX2, OPN, OCN, BSP, TAZ) were analyzed using real-time PCR. The relative mRNA levels of each gene were normalized to β-actin as an internal control. Osteogenic differentiation activities are presented as mean ± SD (n = 3), with statistical significance indicated by $p < 0.05$ and $p < 0.01$.

(B) Protein levels of Col I, OPN, and OCN were assessed by western blotting. Band densities of Col I, OPN, and OCN were normalized to β-actin and represented as arbitrary ratios compared to control titanium.

Conclusion: In summary, our study has shown that bio-functionalizing titanium discs with FN9-10ELP effectively enhances cell adhesion, proliferation, and osteogenic differentiation of human mesenchymal stem cells (hMSCs), maintaining bio-functionality throughout the osteogenic differentiation process. These findings suggest that the sustained biological activity of FN9-10ELP released from titanium over a full 4-week period could enhance bone regeneration and healing, indicating potential utility for implant treatments.

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