Boosting engineered cartilage formation with recombinant spider silk

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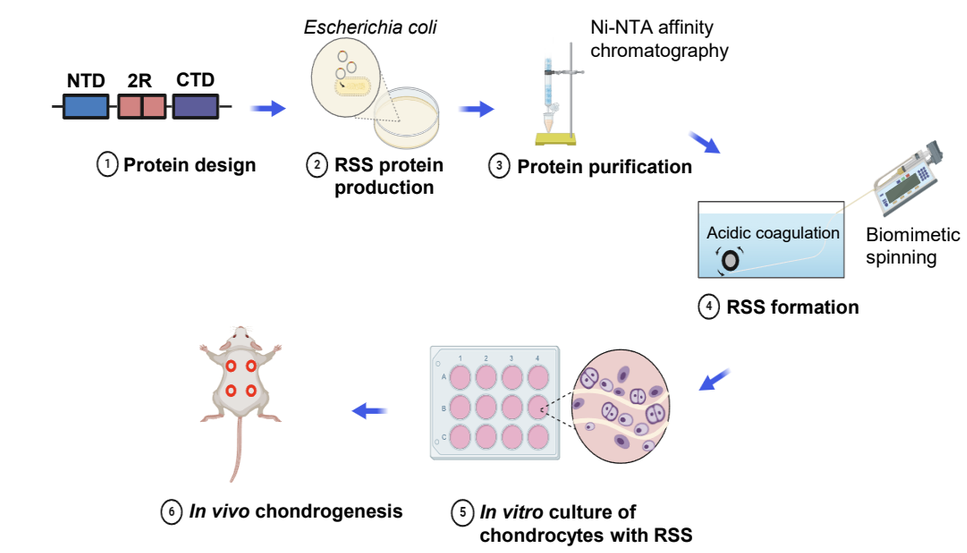
Introduction

Being a tissue of avascular, aneural and lack of lymphatic drainage, articular cartilage has very limited self-regeneration capacity. Discovery of novel therapies for cartilage repair or regeneration remains a significant clinical demand. Generation of functionalized biomaterials that facilitate chondrogenic cell adhesion, proliferation and differentiation is an essential step for functional tissue engineering of cartilage tissue. In this study, a type of newly generated recombinant spider silk (RSS) was examined to modulate chondrocyte fate and to determine its role in fabricating engineered cartilage tissue formation *in vitro* and *in vivo*.

Materials & Methods

RSS structure was characterized by dissection microscopy, SEM, and elemental analysis using EDAX. Primary chondrocytes from neonatal mice were cultured in chondrogenic medium. Extracellular matrix (ECM) proteoglycans and lipid droplets in micromass cultures were visualized with Alcian Blue and Oil Red O staining, respectively. Total RNA was extracted for qPCR and RNA-seq to assess RSS effects on chondrogenic and adipogenic gene expression. For *in vivo* studies, chondrocyte-laden silk-based bioscaffolds were implanted subcutaneously in SCID mice for 3 weeks, followed by Alcian Blue, Safranin O staining and immunohistochemistry staining for Col II to evaluate cartilage formation.

Results



Scheme 1. Fabrication and functional evaluation of RSS

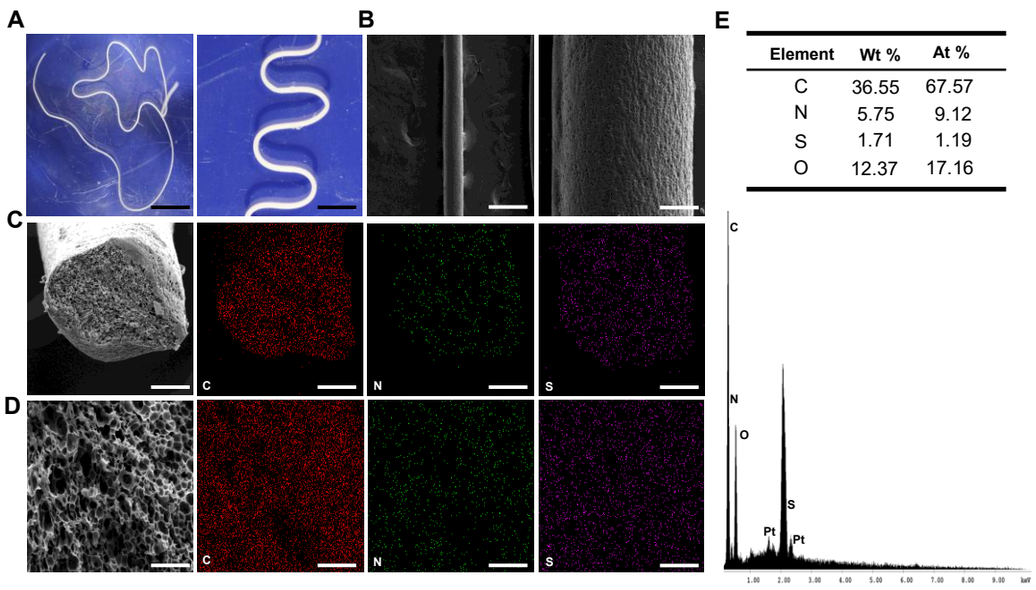


Figure 1. Characterization of RSS. (A) Dissection microscope image of hydrated RSS; (B) SEM top view of freeze-dried RSS; (C, D) SEM cross sections with EDX elemental mapping; (E) EDX analysis showing elemental composition and characteristic peaks. Scale bars: (A) 2 mm, 1 mm; (B) 250 μm, 50 μm; (C) 50 μm; (D) 10 μm.

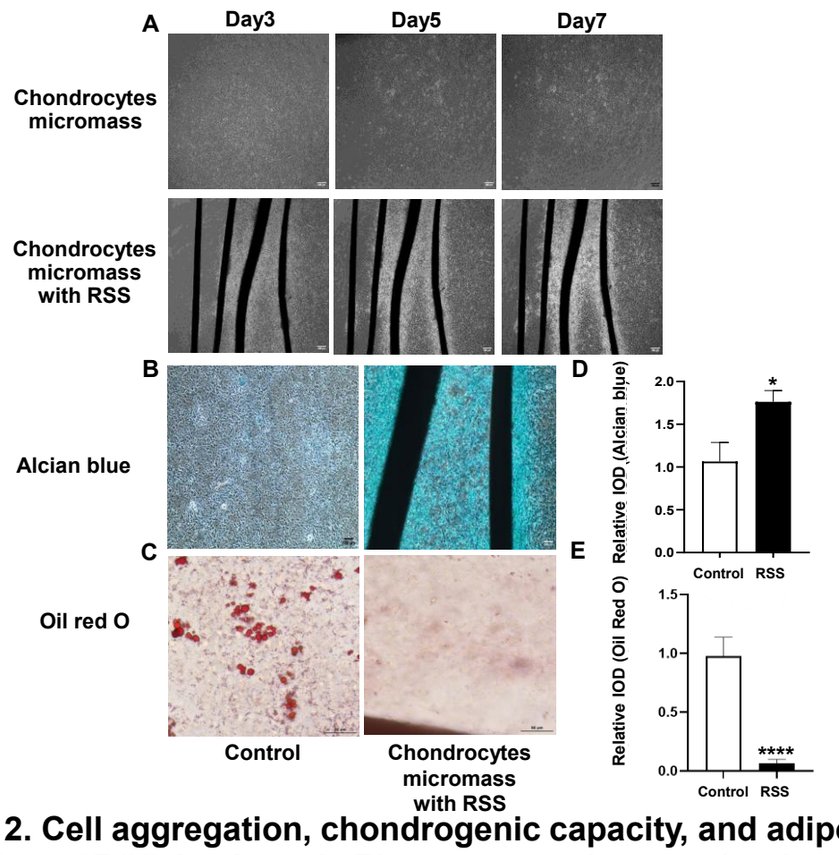


Figure 2. Cell aggregation, chondrogenic capacity, and adipogenesis inhibition of RSS *in vitro*. (A) Phase-contrast images showing that RSS promoted localized cell aggregation and increased cell density; (B) Alcian blue staining images; (C) Quantification of Alcian blue IOD demonstrating increased chondrogenic matrix deposition with RSS; (D) Oil Red O staining images; (E) Quantification of Oil Red O–positive area showing reduced adipogenesis with RSS. (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n = 3; Scale bars: (A) 400 μm, (B) 200 μm, (D) 50 μm.)

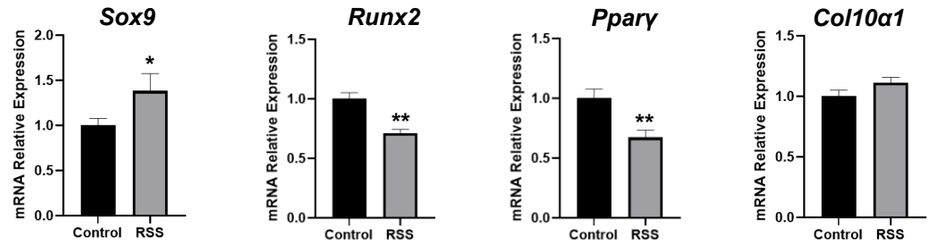


Figure 3. Real-time PCR analysis of chondrogenic and adipogenic gene expression in micromass culture. RSS treatment significantly upregulated Sox9, reduced Runx2 and Pparγ, and did not alter Col10α1, indicating enhanced chondrogenic activity, suppressed adipogenic signaling, and stable hypertrophy-related gene expression. (*P < 0.05, **P < 0.01; n = 3.)

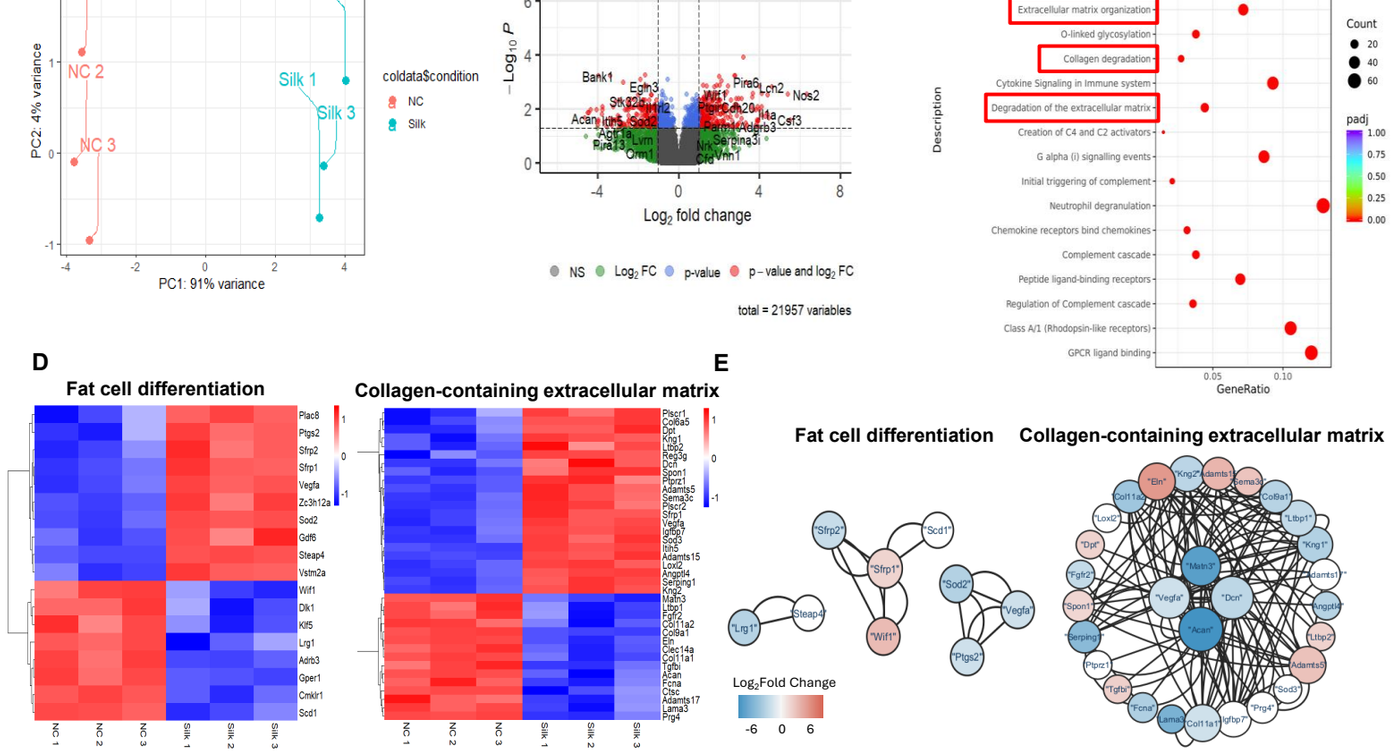


Figure 4. RNA-seq analysis of RSS-treated micromass cultures. (A) PCA showing clear separation between control and RSS groups; (B) Volcano plot of DEGs (≥2-fold); (C) GO enrichment of DEGs; (D) Heatmap and (E) PPI network highlighting genes linked to chondrogenesis and adipogenesis. Results show that RSS influences pathways related to extracellular matrix organization, collagen degradation, and matrix remodeling.

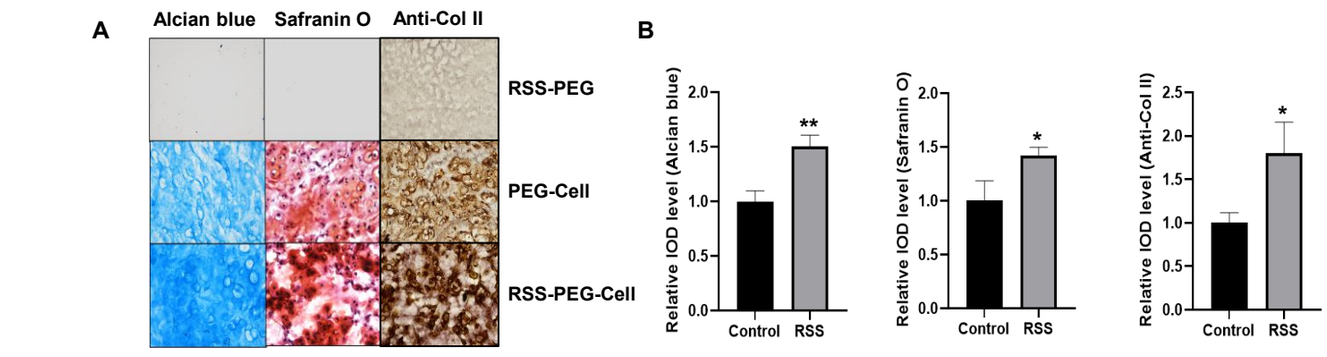


Figure 5. Histological and immunostaining analysis of PEG–cell and RSS–PEG–cell constructs in a subcutaneous mouse model. (A) Representative Alcian blue, Safranin O, and Col II immunohistochemical staining; (B) Quantification of relative IOD values showing significantly higher staining intensity in the RSS–PEG–cell group. These results indicate that RSS enhances chondrocyte phenotype maintenance and ECM deposition, promoting neocartilage formation. (*P < 0.05, **P < 0.01; n = 3; Scale bars: 100 μm.)

Conclusion

The synthetic RSS possesses extraordinary properties including excellent biocompatibility, non-toxicity, slow biodegradation, and chondrogenesis supportive, making them an ideal matrix material for engineered cartilage tissue formation that can be potentially used for cartilage repair or regeneration.