

## ENGINEERING

## Healing Osteoarthritis: Engineered Proteins Created for Therapeutic Cartilage Regeneration

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Millions of people worldwide are afflicted with painful osteoarthritis, which is characterized by degradation of articular cartilage found in major joints such as the hip or knee. Symptoms include inflammation, pain, and decreased mobility. Because cartilage has a limited ability to self-heal, researchers have focused efforts on methods that trigger cartilage regeneration. Our approach is to develop an injectable, protein-based hydrogel with mechanical properties analogous to healthy articular cartilage. The hydrogel provides an environment for cell growth and stimulates new tissue formation. We utilized recombinant DNA technology to create multifunctional, elastomeric proteins. The recombinant proteins were designed with biologically active domains to influence cell behavior and resilin structural domains that mimic the stiffness of native cartilage. Resilin, a protein found in the wing and leg joints of mosquitoes, provided inspiration for

the mechanical domain in the recombinant protein. The new resilin-based protein was expressed in *E. coli* bacteria. Forming hydrogels requires a large quantity of engineered protein, so parameters such as bacterial host, incubation temperature, expression time, and induction method were optimized to increase the protein yield. Using salt to precipitate the protein and exploiting resilin's heat stability, 27 mg/L of recombinant protein was recovered at 95% purity. The protein expression and purification protocols were established by analyzing experimental samples on SDS-PAGE gels and by Western blotting. The mechanical properties and interactions with stem cells are currently being evaluated to assess the potential of the resilin-based hydrogel as a treatment for osteoarthritis.

Research advisor Julie Liu writes, "There is increasing interest in using adult stem cells in tissue engineering therapies for damaged cartilage. Our overall goal is to understand how material properties and the local environment can be used to control stem cell differentiation so that we can engineer safe and effective cartilage replacements."

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