Genetically Expanded Collagen for Tissue Engineering

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Collagen is an essential structural protein in the extracellular matrix, which provides physical and biological support for cells. In tissue engineering, collagen is utilized due to its biocompatibility, biodegradability, and cell support properties. Usually, collagen is extracted from animal tissues, but concerns have emerged over transmission of diseases. Research has recently focused on producing recombinant collagen in bacterial or plant systems. It is possible to produce engineered collagen-mimicking polypeptides in *E.coli* that self-assemble to form collagen fibrils, but these polypeptides do not possess post-translational modifications.

Our research goal is to incorporate L-3,4-dihydroxyphenylalanine (L-DOPA) into collagen via the genetic code expansion method. L-DOPA can enhance cellular attachment, due to a catechol side chain that enhances adhesion.

We have created an *E.coli* system, which produces collagen-mimicking polypeptides that contain L-DOPA, at specified locations. The peptides have a triple-helix structure that can self-assemble in-vitro under optimal conditions to form collagen fibrils. Further, we have shown that L-DOPA presence enhances the fibrillation process. Cultured fibroblast cells in the presence of our peptides in culture medium remain viable, ruling out peptide toxicity. 2-D films composed of peptides containing L-DOPA mixed with alginate supported cell adhesion and spreading to a better extent compared with films with peptides w/o L-DOPA.

This innovative approach combines two cutting-edge technologies, tissue engineering and synthetic biology, while creating novel biomaterial by genetically incorporating L-DOPA into collagen. This research is expected to greatly impact the regenerative medicine field, as we will generate a generic biomaterial able to support various cells.