Production and Biocompatibility of Spider Silk Proteins in Goat Milk | Biological Engineering

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Doctoral Dissertation Defense
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Full Abstract

Due to its biocompatibility and impressive mechanical properties, spider silk has great potential for a variety of commercial applications, from biomaterials to textiles. Unfortunately, it is difficult and impractical to obtain native spider silk in sufficient quantities to fully investigate these applications. In light of this, recombinant spider silk proteins have been produced in a variety of hosts, including microbes, plants, silkworms, and goats. While these recombinant proteins have potential for meeting the demands associated with investigating spider silk's impressive properties more fully, each of the current production methods needs to be improved upon in one way or another. Currently, goats produce the highest consistent yields of recombinant proteins, but there is room for improvement in both production capacity and protein length. The first focus of this dissertation was to establish a goat cell line designed to increase the purity and quantity of recombinant proteins produced in goat milk by incorporating a spider silk gene that encodes for a histidine-tagged protein into the goat genome. Although multiple genomic integration techniques were investigated, the PiggyBacTM Transposon Vector System was ultimately used to successfully establish a new “spider goat” cell line.

The second focus of this dissertation was to investigate the biocompatibility of materials made from the current goat-derived recombinant spider silk proteins. Prior to testing the biocompatibility of these proteins, a method was developed for destroying endotoxins in the proteins that had been introduced during milk collection and downstream processing. Two in vivo biocompatibility studies were conducted in rats by implanting two different silk materials that had been treated using the endotoxin destruction method established herein. In response to the low level of biocompatibility discovered during these studies, another in vivo study was conducted using materials made from goat-derived proteins that had been purified using reverse phase chromatography. It was determined based on results from this final study that goat-derived spider silk proteins are not biocompatible in their current state due to impurities in the proteins and that further work will need to be done to improve the protein purity.