Together with undergraduate students, Dr. Todd Johnson from the chemistry department and Dr. Barbara Trask from the zoology department are proposing a collaborative project to develop novel techniques using relatively inexpensive reagents and equipment to quantify the amount of collagen in mouse skin. Working with a zoology student this past summer, Dr. Trask began characterizing a strain of transgenic mice in which a gene that has been shown to affect the normal production and export of collagen has been deleted. They found that dermal wounds in these genetically altered mice healed at a rate different from those in normal mice. She would like to continue this work investigating the nature of the scar formation in the healed wounds of these genetically altered mice and compare it with the scar tissue in normal mice.

While there are greater than twenty different forms of collagen, when grouped together, the collagens represent the most abundant extracellular protein in all mammals, including humans. Indeed, collagen makes up approximately 25% of the total protein content in the human body. As one would predict, alterations in such an abundant protein—either in its structure or its abundance—lead to physiologic abnormalities. For example, mutations in the gene for collagen (type I) result in *osteogenesis imperfecta*, a disease marked by abnormally brittle bones. Additionally, the over-production of collagen in wounded skin results in scar formation and, when excessive and persistent production of scar tissue occurs in other tissues, the result is fibrosis. Fibrotic disease is associated with organ failure in many different tissues such as lung, kidney, heart and liver.

Given the abundance of collagen in skin, as well as the importance of this protein in wound healing and scar formation, we will need to identify a way of quantifying the collagen in this tissue.

Although each of the collagen proteins performs slightly different biological functions and/or is located in slightly different places, all have some structural elements in common, including the post-synthetic addition of a chemical group called a hydroxyl group. This modification only takes place on one particular type of amino acid called proline. This modification of proline to hydroxy-proline, is unique to members of the collagen protein family and provides a method by which the quantification of collagen within a mixture of other proteins, such as is found in whole tissues, can be made. Deficiency in vitamin C results in the inability to convert proline into hydroxy-proline. The net result of this slight alteration in the structure of collagen leads to a connective tissue disorder we call scurvy. Currently, collagen content from tissue samples is determined by measuring the proportion of regular proline to hydroxylated prolines using an amino acid analyzer. This technology is costly and requires expensive, specialized equipment. We are proposing a collaborative project to develop novel techniques, using relatively inexpensive reagents and equipment, to quantify hydroxylated proline in the skin of transgenic and normal mice. The outcome of this research will likely be generally applicable in the understanding of wound healing and fibrosis. There are numerous pathologies associated with abnormal collagen in humans and a better understanding of how this missing gene affects wound healing in the transgenic mouse model may lead to significant breakthroughs in the areas of wound and bone healing in humans.

BUDGET ITEM	COST
Zoology (Animal Cages and husbandry items [bedding, food, etc.], tools for surgical excision of mouse tissue, chemicals for tissue preparation, extraction vessels and freeze-drying vials)	~$2000-2500
Chemistry (chromatography columns, solvents, reagents for chemical reaction with hydroxyproline, chemical standards [hydroxyproline and collagen])	~$2500
Commercial Company TBA (quantification of collagen concentrations in some samples to verify the accuracy of the newly developed technique relative to standard methods of quantification)	~$1000
Student Stipend: 2 students, 1 each in chemistry and zoology for 5hrs/week x30weeks @ $10/hr + Benefits @ 8.5%	$3255
GRAND TOTAL	$9255