Proposal Requesting Summer 2010 Research Support from the Baker or Rose Hills Foundations

Richard C. Haskell, Professor of Physics

Immuno-Gold Labeling of Cell Phenotype in Engineered Corneal Tissue

Requested funds – 10 weeks of student research ($5K)
plus $1K for equipment and supplies = total of $6K

Baker or Rose Hills – I would prefer Baker Foundation funds to support one of the current research students in my OCM lab, but if only Rose Hills funds are available, Perry Ellis (HMC junior physics major) has expressed interest and is eligible for Rose Hills funds.

Proposed Research

Overview: The HMC tissue-engineering group, led by Prof. Orwin, is engaged in a multi-faceted research program aimed at the development of a human corneal tissue replacement. Over ten million individuals worldwide experience corneal blindness, and corneal transplants are currently the only treatment for restoring vision. Corneal transplants have a 90% success rate in patients with good prognoses, but almost no chance of success in patients with alkali burns or recurrent graft failures. The shortcomings of corneal transplantation include significant immune rejection rates, the possibility of infections, and donor shortages. A tissue-engineered corneal replacement will avoid the possibility of immune rejection and circumvent the current shortage of corneal donors.

The ultimate goal of the HMC cornea research program is to produce a normal corneal stroma tissue with native, ordered ultrastructure that renders the tissue transparent in the visible spectrum. One key component of this research program is the visualization of corneal tissue as it develops in sterile culture. The HMC optical coherence microscope (OCM) group, led by Prof. Haskell, is collaborating with the tissue-engineering group in the development of a novel immuno-gold labeling technique to help distinguish corneal cells from the collagen matrix, and to probe non-invasively and *in situ* the phenotype of corneal cells during culture. In effect, we would like to attach gold nanoparticles, 90 nm in diameter, to antibodies that bind to specific proteins on the surface of the cell. The presence of these membrane proteins is correlated with specific cell behavior, so the binding of gold nanoparticles to a particular cell indicates the behavior or “phenotype” of the cell. These bound gold nanoparticles are readily visible in images acquired with an optical coherence microscope (OCM), and will “light up” cells with a specific phenotype, even deep within a tissue. The OCM group has found that the OCM is uniquely capable of imaging corneal tissue in sterile culture throughout its millimeter thickness. We think

that the ability to monitor and ultimately to control cell phenotype in these corneal tissue cultures is critical to achieving a viable corneal tissue replacement.

Recent achievements: Over the past year we have succeeded in attaching secondary antibodies to gold nanoparticles via polyethylene glycol (PEG) molecules, and these "PEGylated" gold nanoparticles are clearly visible in OCM images. Moreover we have shown that the PEGylated gold nanoparticles attach to primary antibodies that are bound to corneal cells exhibiting a particular phenotype. These bound PEGylated gold nanoparticles do indeed "light up" cells expressing this specific phenotype in OCM images. Unfortunately, the PEGylated nanoparticles also bind nonspecifically to other proteins in the cells, spoiling the otherwise unique highlighting of cells expressing the specific phenotype. (See Fig. 1 below.) Our goal for the summer of 2010 is to eliminate the nonspecific binding and obtain OCM images of clearly and uniquely labeled corneal cells that are exhibiting a specific phenotype, and to correlate these images with measurements of cell phenotype using molecular biology assays. If successful, these results will serve as a compelling demonstration of the immuno-gold labeling technique and will serve as the basis for our first manuscript describing this work.

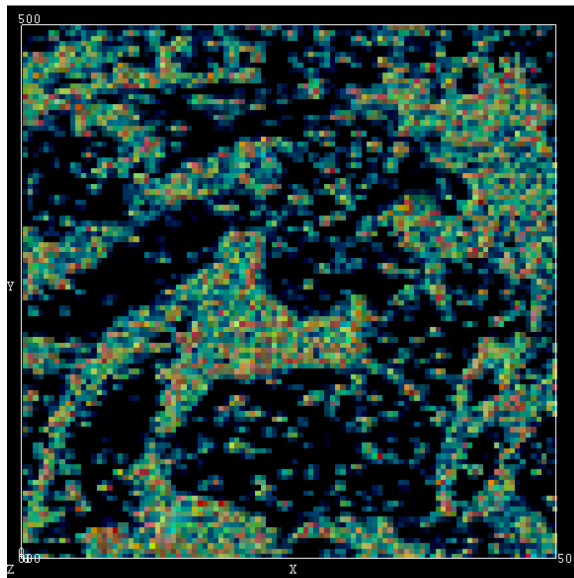

Figure 1. Recent OCM image (500 x 500 micrometer field of view) of a monolayer of cells cultured on a coverslip, and immuno-labeled with PEGylated gold nanospheres. The bright green-to-red pixels are the PEGylated gold nanospheres adhering to the cells which nearly but not completely cover the surface of the cover slip (dark blue-black). While the cells are certainly labeled, the gold nanospheres are adhering to some parts of the cells that do not contain the targeted integrin receptor – this is the so-called "nonspecific binding". We must reduce or eliminate the nonspecific binding to be sure that we are getting an accurate highlighting of cells expressing a particular phenotype.

Student measurements and activities: The day-to-day tasks performed by students fall into roughly three classes.

(1) The detailed molecular structure of the PEGylated gold nanospheres can be probed with dynamic light scattering (DLS) measurements of the hydrodynamic radii of the nanospheres. (The DLS instrument is generously provided by Prof. Baker and is housed in her Jacobs lab.) By varying the steps of successive molecular bindings to the gold spheres and measuring the hydrodynamic radii of the resulting gold spheres, students will be able to infer the structure of the spheres. We anticipate that students will be able to tweak the structure of the PEGylated gold nanospheres and also vary the type of antibodies used to form these structures with the ultimate goal of reducing nonspecific binding. (The biochemistry involved in preparing PEGylated gold nanospheres will be performed in Prof. Haskell's OCM lab suite in the sub-basement of Keck.)

(2) Students will culture corneal cells in monolayers on cover slips and also on collagen substrates on cover slips. Corneal cells will also be cultured on 3D collagen scaffoldings to produce thick (1 mm) opaque tissue cultures. These samples will then be immuno-labeled with PEGylated gold nanospheres, varying the structure of the nanospheres and the labeling procedure to minimize nonspecific binding. (Cell cultures will be performed in Prof. Orwin's tissue-engineering labs in Parsons.)

(3) The various types of cell cultures will be imaged with OCM and with confocal fluorescence microscopy. (The Zeiss confocal microscope is housed in the basement of Olin.) The confocal images will be analyzed qualitatively and the OCM images will be analyzed quantitatively to determine whether the nonspecific binding has been reduced or not.

Context of student activities: This is truly an interdisciplinary project. The five students and two faculty involved in this project will meet several times a week as a group to discuss progress and address obstacles. In past years we have observed and encouraged the type of multidisciplinary collaboration that is necessary in this type of research, and have found that it can be extremely synergistic and productive. Students will be encouraged to submit their work on this project to local and national conferences, exposing them to presentation skills and cutting-edge research in their field, which will be invaluable as they pursue graduate careers in research.

Significance of proposed research: We are very close to achieving success in this immuno-gold labeling of cultured corneal cells. By labeling cells in 3D tissue cultures, we hope to follow the phenotype expressed by cells as we try a variety of chemical and mechanical stimuli to control the phenotype of cells and hence their transparency in the visible spectrum. Only transparent tissue cultures can serve as potential human corneal replacements. These tissue-engineered corneal replacements can be generated with cells from the recipient's own body, avoiding the complications of immuno-rejection of the corneal replacement that has plagued other types of artificial corneas. The sight of millions of people world-wide can be restored with a successful tissue-engineered cornea

replacement. The number of people in need of new corneas is growing as the population ages and procedures such as LASIK become more commonplace.

Impact of the Proposed Research on the HMC Community

Student response to the cornea project has been enthusiastic – even passionate! The mission of the project elicits the best in Mudders – a genuine desire to contribute to the welfare of others and society in general. This type of positive energy is at the heart of HMC strategic goal (5) Global Engagement and Informed Contributions to Society. The multidisciplinary nature of the project also appeals to students and requires strong communication and collaboration among researchers, giving rise to a vibrant social group spirit. These are the ingredients of HMC strategic goal (2) Focus on Experiential and Interdisciplinary Learning, and a little bit of goal (4) Nurturing and Developing the Whole Person.

The cornea project has direct connections to the existing biophysics half-course, Physics 174 – Introduction to Biomedical Imaging, and to the biomedical engineering course, Engineering 164 – Introduction to Biomedical Engineering. In fact, student participation in cornea research has become the primary career pathway for Mudders to the rapidly growing areas of biophysics and biomedical engineering. Over 200 students have participated in the OCM and cornea labs over the past 15 years, creating a pocket of activity that has influenced other students to venture into these areas after graduation.

During the summer of 2003, the OCM/cornea project employed students from all majors in the College: physicists, engineers, and biologists were obviously needed, but mathematicians and computer scientists were also engaged for image reconstruction and some image analysis, and chemists and biochemists prepared the colloidal gold solutions and performed the molecular biology assays. The sheer breadth of the science and math engaged in the research ensures that the project impacts the entire HMC community.

Prof. Orwin's lab is fortunate to have partial support from the Engman Funds, and recently Prof. Orwin was awarded an NIH AREA grant for the cornea project. Prof. Haskell was a co-principal investigator on the NIH grant proposal, but asked for no funding for the OCM lab to minimize the budget and maximize the probability of success for the proposal. If the current proposal for HMC internal funds to support the OCM summer research program is successful, we should be able to generate convincing preliminary results that will enable Prof. Haskell to compete successfully for NSF funding. The OCM project benefitted in the past from two large NSF grants spanning roughly six years. We aim to pursue another infusion of cash!!