

UROP Summaries

Microfluidic device for rapid isolation of pure leukocyte populations

June 2002 – August 2003

Massachusetts General Hospital- Center for Engineering in Medicine

Principal Investigator: Dr. Mehmet Toner

Supervisor: Dr. Sethu Palaniappan

Melis Anahtar, Class of 2008

Majors: Mechanical Engineering and Brain and Cognitive Science

The entire human circulatory system contains only enough purified leukocytes to fill a 2oz. shot glass. This small cell population defends the body against disease and provides invaluable clinical information regarding a person's physical condition. Leukocytes are accompanied in the blood stream by erythrocytes and platelets, pieces of megakaryocyte cytoplasm. Neither of these bodies contain DNA, nor permit clinical information extraction. Upon obtaining a phenotypically homogeneous leukocyte population by removing the erythrocytes and platelets, we can analyze the leukocyte's gene expression through detailed molecular analysis to predict human response to an immunoinflammatory injury.



Figure 1. The schematic design of the microfluidic device.

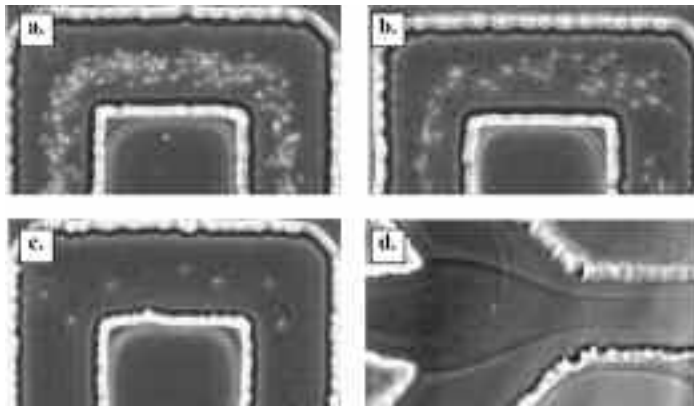


Figure 2. A series of images of a trial using 1:100 diluted blood and Zap-Oglobin lysing solution taken using microscopy. The first serpentine channel (a) has a stream containing white blood cells with high concentrations of red blood cells. As the lysing solution diffuses into the cell stream, red blood cells are lysed (b). The lysed red blood cells are seen in black, the unlysed cells are white. In the final serpentine (c), only white blood cells are left since the lysing solution has had enough time to diffuse and get rid of nearly all of the erythrocytes. The outlet (d) shows the quenching-using isoton to prevent lysing in the tube. Laminar flow, which occurs as a result of low Reynolds numbers in microfluidic devices, is clearly shown in (d). The liquid diffusion into the cell stream is significantly faster than the cell diffusion into the lysing stream.

The goal of this project is to create a microfluidic device that isolates leukocyte populations by lysing erythrocytes in a minimum amount of time. The device was produced in a microfabrication facility using conventional soft lithography and tested using different types of blood, lysing solutions, and quenching solutions. Qualitative data shows that an approximately 97 percent pure leukocyte population is produced by the device in around three seconds. The device is a great improvement upon current leukocyte isolation tech-

niques and has powerful applications in the laboratory and medical field. Its main advantage is a lack of reliance upon a specific cell size, allowing leukocytes to be isolated from an assortment of blood types. The leukocytes are also exposed to nearly identical environments as a result of the uniformity and high level of control experienced at the microscale, preventing *ex vivo* gene activation.

Adapting a typical handheld device for Global Positioning System (GPS) tracking within MIT

March 2004 – Present

Clinical Decision Making Group – MIT CSAIL

Principal Investigator: Dr. Peter Szolovits

Mentor: Bill Long

Ojonimi Ochoi, Class of 2005

Major: Electrical Engineering and Computer Science

Minor: Biomedical Engineering

The purpose of this project is to accurately record a patient's physical activity level and to enable both doctors and patients to make more informed decisions about their health. For older adults and patients with chronic illnesses, it is especially important to exercise moderately each day. The activity level has traditionally been measured by the number of steps the individual has taken. A device called a pedometer is used to count the steps and monitor hip motion experienced during walking.

Current devices, however, cannot differentiate between the bounces experienced while running from those experienced when driving a vehicle. In addition, they cannot record aquatic exercises if the subject does not strap on the appropriate device. This results in inaccurate data accumulation.

My task is to modify a typical handheld device (a Linux-based HP Ipaq) for GPS tracking within MIT to improve accuracy. In order to adapt the device, I am devising a way to interpret the information from the GPS device on the Ipaq, and then writing a program to provide an interface between the two. In addition, I will download freeware maps of MIT into the device and add the needed features to help determine locations more accurately. Thus, the device would be able to pinpoint and track the location of an individual and measure his or her activity level. I hope this project will help provide services to the needy.

Studies of how radiation-induced adaptation modulates toxicity and homologous recombination

January 2005 – Present

Division of Biological Engineering

Principal Investigator: Professor Bevin P. Engelward

Scot G. Frank, Class of 2008

Major: Biology, Electrical Engineering and Computer Science

Minor: Biomedical Engineering

One in three people develop some form of cancer, and of those, half are treated with radiation. The tumorigenic cells composing cancers develop from the accumulation of mutations to those genes that control cell growth and division. Errors in the DNA arise from un- or mis-repaired damage caused by radiation, free radicals, chemicals, and other sources. A type of DNA lesion that is particularly deleterious to cells occurs when both strands of a DNA duplex are broken, forming a double stranded break (DSB). Homologous

recombination (HR) is one way in which cells can repair DSBs. HR utilizes segments having sequence similarity within the genome to restore bases lost at a DSB (Figure 1). After a break occurs, the strand is sectioned to produce 3' overhangs, homologous sequence is searched, and replication begins replacing the missing sequence. Holliday junctions are then resolved resulting in either crossover or no crossover of flanking sequences. This process occurs about ten times every during every cell division. If this is a harmful mutation, the cell may be adversely affected when the intact strand copies this mutation to the broken strand. Accumulation of many such mutations in a single cell can cause uncontrolled cell growth and proliferation, or cancer.

In tissues exposed to an acute dose of radiation, the rate of HR is greatly increased in effort to repair the resulting damage. An increase in HR may increase the chance of deleterious recombination events such as insertions, deletions, translocations, and loss of heterozygosity. Recently, a strain of fluorescent yellow direct repeat mice (FYDR) mice has been created at MIT that make it possible to directly detect recombinant cells by fluorescence. Using these mice, it was shown that the level of HR decreases in animals chronically exposed to radiation. This elucidates a still obscure relationship between exposure length and dosage. Since HR modulates the toxicity of many cancer chemotherapeutics, this result may clarify the mechanism of current combinatorial radiation-chemotherapy treatment practices. A decrease in HR indicates different repair pathways are being utilized to combat the DNA damage caused by radiation. Therefore, prescribing chronic exposure to ionizing radiation may in fact increase our body's susceptibility to recombinogenic chemotherapeutics through the decreased activity of HR.

The aim of this research is to explore the relationship between chronic exposure to radiation and the sensitivity of cells to chemotherapeutics. We hope to reveal how clinical doses of radiotherapy modulate sensitivity of tumor cells to killing by chemotherapeutics.

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Directing embryonic stem cell differentiation through RNA interference

Fall 2004 - present

Department of Chemical Engineering

Principal Investigator: Prof. Robert Langer

Supervisor: Dr. James Lu

Tiffany Chen, Class of 2006

Majors: Electrical Engineering and Computer Science, Biology

Minor: Biomedical Engineering

My project was to investigate methods for directing differentiation of murine embryonic stem (ES) cells to osteoblast and dendritic cell types through RNA interference. This knockdown of gene function can be accomplished by introducing translation blocking short interfering RNA (siRNA) sequences or short hairpin RNA (shRNA) constructs that can be diced into siRNA. Lipofection of a GL3-firefly luciferase plasmid has successfully induced luminescence in mouse ES cells. However, co-transfection of dual luciferase

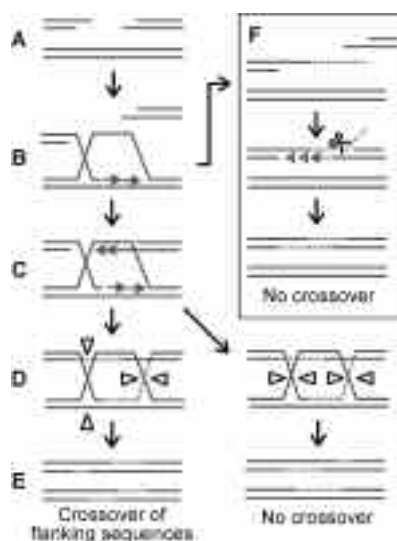


Figure 1. Mechanisms of DNA recombination.

(renilla and firefly) DNA with anti-firefly luciferase siRNA has not only resulted in high renilla luminescence, but also high firefly luciferase luminescence levels. Currently, we are experimenting with a DNA vector-based shRNA construct that targets GL3-firefly luciferase. Once a high transfection efficiency of RNA has been established, we can modify the function of genes known to be important in stem cell differentiation, such as OCT4 and members of the bone morphogenetic protein family. This would enable a direct genetic differentiation to certain cell types.

Investigations of biochemical mechanisms of inflammation in endothelial cells as a result of advanced glycation end-products

June 2004 to August 2004

Department of Chemistry, Santa Clara University (NSF REU Program)

Principal Investigator: Dr. Ram Subramaniam

Victoria Chang, Class of 2006

Major: Chemistry

Type-II Diabetes is a non-insulin related disorder which results in hyperglycemia, an excess of glucose and glucose derivatives in the blood. This hyperglycemia and its side effects cause inflammation, a usually normal response to infections. However, hyperglycemia-induced inflammation may be severe enough to result in many of the complications of diabetes, including atherosclerosis and kidney damage.

It is the carbonyl group of the glucose and glucose derivatives that interact with proteins. Certain amino acids, lysine and arginine, contain an amine in their side chains. If accessible, the aldehyde of the glucose and the amine side chain will undergo a non-enzymatic reaction which leads to the formation of a Schiff base. The Schiff base then undergoes further irreversible modifications to form an Advanced Glycation End-Product (AGE).

We studied the mechanism and pathway by which AGEs cause inflammation. We hypothesized that it involves the interplay between monocytes and endothelial cells. Both monocytes, part of the body's immune system, and endothelial cells, the flat cells that line the blood vessels, are in constant contact with blood, and, in the case of diabetes, are exposed to AGEs. Monocytes are a key player in the inflammatory response, and endothelial dysfunction is linked with many of the complications of diabetes.

We tested the response of Human Umbilical Vein Endothelial Cells (HUVEC) and THP-1 Monocytes to hyperglycemia and AGEs. Our preliminary results showed that the HUVEC released PGE₂, a prostaglandin involved in the inflammatory pathway, in response to treatment with AGE but not unmodified proteins. We also found that HUVEC did not independently release TNF- α — an inflammatory cytokine — or vascular cell adhesion molecules in response to AGEs, high glucose, or a positive control. Current research is testing the response of THP-1 to AGE and hyperglycemia. In addition, the inflammatory response of HUVEC under conditions of interaction with THP-1 is also under investigation.

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Integrated radiotherapy imaging system for image guided and gated therapy

December 2004 - Present

Department of Radiation Oncology, Division of Radiation Physics
Massachusetts General Hospital

Principal Investigator(s): Professor George T. Y. Chen, Dr. Gregory C. Sharp

Yin Ren

Major: Electrical Engineering and Computer Science

In Radiation Therapy, a lethal dose of radiation in the form of high energy (4-25 million volts) X-ray photons is delivered to the tumor, while the dosage to surrounding healthy tissues is minimized. Recent technical developments, such as Intensity Modulated Radiation Therapy (IMRT), allows physicians to precisely deliver radiation to 3D volumes by irradiating different areas for different lengths of time. However, internal organ motion during treatment, mainly caused by respiration, can greatly degrade the efficiency of radiotherapy. In the case of tumors located in the thorax and abdominal regions, tumor movement due to breathing can complicate delivery of radiation.

One solution to the problem is to accurately track tumor motion in real time, and deliver radiation only when the tumor moved to certain positions during the respiratory cycle. An imaged guided system, known as Integrated Radiotherapy Imaging System (IRIS), is being developed for this purpose. IRIS consists of a pair of diagnostic X-ray tubes and flat-panel detectors, mounted on the gantry of a Varian Clinac® 21EX Linear Accelerator. The imagers can be used to provide X-ray data through simultaneous radiography and fluoroscopy (a continuous series of 'live' X-rays), and track the motion of tumors, through external fiducial markers or internal anatomy such as the diaphragm.

I have been working on a system known as the Real-time Position Management (RPM). By placing an external marker on the patient and through monitoring respiration, RPM allows the treatment to be "gated" by the patient's respiratory cycle. In order to connect the IRIS and RPM system, my task is to modify and design a prototype on a breadboard, which communicates between the X-ray imagers, the linear accelerator, the RPM system, and



Figure 1. The IRIS system. Two X-ray imagers are mounted on the gantry of the Linear Accelerator and are orthogonal to each other.

control switches. I first read about various components in the circuit and understood how they worked together, then proceeded to design my own to include additional features. Working with engineers from Varian Medical Systems, I was able to synchronize the X-ray units and the linear accelerator treatment beam, by adding circuitry that delays the delivery of treatment until the imagers acquired their image data. I also added a new switching feature that enables the physician to easily switch between "Gated" IRIS and traditional Fluoroscopy treatment modes, and an additional safety feature in IRIS mode. Taking the breathing data from the RPM system, I was able to trigger the delivery of radiation based on amplitude gating of respiration, which is the first step towards gated radiotherapy. Right now, I have been working on including more versatility by allowing direct communication between the two systems, adding more features to the control circuit, and making PCB designs for manufacturing.

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