

# REVIEWS

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## A Survey of Bioengineering Research in Canada-2007

Andrew J. Daugulis

Dept. of Chemical Engineering, Queen's University, Kingston, ON, Canada K7L 3N6

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*Research activity in bioengineering at Canadian universities has been surveyed. Details were provided by chemical engineering departments in response to a common request for information on activities by individual researchers and for key publications. The information provided has been grouped by topics within the broad theme of "Bioengineering," and contributions from individual departments have been summarized within these topics. Although many aspects of bioengineering research are being pursued in Canada, it would appear as though environmental biotechnology, biomaterials, and tissue/cell culture are the most active areas under investigation.*

*Keywords: bioengineering, research, Canada, academic*

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### Introduction

In response to a request by Drs. Arin Sen, University of Calgary, and Hasan Uludag, University of Alberta, coinciding with their coordination of Bioengineering topics at the 57th annual Canadian Society for Chemical Engineering conference held in Edmonton Alberta in October 2007, I have undertaken a survey of research activities in bioengineering in Canada. In an e-mail sent to chairs/heads of chemical engineering departments across Canada in June 2007 (with a reminder in August), information was requested on the research activities of individual faculty members, and a call for a few key publications highlighting the research summaries. The request was also sent to a few nonchemical engineering university departments undertaking related research, and to the Biotechnology Research Institute of the National Research Council of Canada in Montreal. Those participants of the 57th CSChE conference who were contributing research papers to this special edition of *Biotechnology Progress* were also invited to provide research summaries. Private sector organizations were not contacted because of concerns that information would not be readily forthcoming due to proprietary issues. Responses were received from virtually all who were contacted, and for the most part they were prepared by individual faculty members themselves. The responses were then compiled into somewhat arbitrary themes, and edited for brevity and consistency of style. Contributions from departments within a specific research area were subsequently grouped together due to some occasional overlap in focus and collaborations within a department. Where individuals were undertaking research on more than

one topic, their activities were placed into the appropriate area(s).

Linked to research in bioengineering, a number of related undergraduate initiatives were also described in some of the responses; however, the information that was provided was somewhat cursory and incomplete (not all departments sent contributions on this topic) and has not been included in this compilation. A review of undergraduate bioengineering initiatives may be a worthwhile future activity. I fervently hope that I have not misrepresented any of the submissions sent to me, or summarized/simplified to the point of falsification. I do hope that my colleagues in Canada, and the larger bioengineering research community, will find this survey to be of interest.

### Environmental and Bioresource Engineering

At the University of Toronto, Elizabeth Edwards' laboratory is focused on biodegradation and biotransformation of pollutants in surface water and particularly in groundwater. Edwards characterizes anaerobic microbial communities that degrade pollutants and designs nucleic acid probes and DNA microarrays to identify metabolic pathways and to detect activity and gene expression in environmental samples.<sup>1</sup> She collaborates extensively with environmental consultants to develop bioremediation and bioaugmentation strategies for chlorinated solvent remediation that have been brought to the market. Her demonstration that benzene could be oxidized anaerobically is now recognized as an important component of natural attenuation processes at hydrocarbon-contaminated sites.<sup>2</sup> Emma Master's research also at Toronto complements the bioenvironmental program with an emphasis on bioconversion of lignocellulosic biomass to renewable materials and fuels. A principle aim of Master's research is

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Correspondence concerning this article should be addressed to A. J. Daugulis at [andrew.daugulis@chee.queensu.ca](mailto:andrew.daugulis@chee.queensu.ca).

to develop and apply enzymes from plants and microbes for the creation of value-added products from wood fiber.<sup>3</sup> Master's laboratory also investigates anaerobic bioconversion of pulp mill residuals to methane. Grant Allen's research in bioprocessing and biological waste treatment combines process engineering and biomolecular methods to enhance and expand the application of wastewater treatment of bioactive pollutants and the biofiltration of air pollutants.<sup>4</sup> For example, the effects of commonly encountered temperature shifts on biological treatment of pulp mill effluents have been extensively characterized leading to improved operation.<sup>5</sup> More recently, Allen's research is making use of bioprocesses to convert wastes into value-added fuels and chemicals. Also at Toronto, Brad Saville's research interests in bioprocess technology emphasize the performance, characteristics, and applications of enzymes for biofuels, starch processing, pharmaceuticals, and in pulp and paper processing. This includes novel glucoamylases and alpha-amylases that are being used commercially for ethanol production, and a recombinant form of oxalate oxidase for industrial and therapeutic application. Ramin Farnood's interests are in applying surface science concepts to environmental problems in measuring the mechanical properties of bioflocs and the implications on adequately disinfecting drinking and wastewater. Levente Diosady's group at Toronto is developing technologies for the food industry focusing on fortification with iron and iodine for the prevention of micronutrient deficiency disorders. Related research is focused on edible oilseed processing, including oil hydrogenation and refining, protein isolation and purification, and recovery of valuable minor ingredients.

Murray Gray at the University of Alberta is studying microbial attachment to oil-water interfaces and the transport of compounds for bioremediation applications. His group has determined that membrane transport in a strain of *Pseudomonas* with <sup>13</sup>C labeled PAHs was an active process, whereas uptake was by passive diffusion through the cell wall.<sup>6</sup> The active efflux system had significant homology with multidrug efflux pumps, likely evolving as a general protective measure that reduced the potential effectiveness of bacteria for bioremediation. The study of cell attachment to interfaces is also underway, along with the use of atomic force microscopy (AFM) to explore the morphology of soft, living cells in aqueous buffer, to map bacterial surface heterogeneities, and to directly correlate the results in the AFM force distance curves to the macroscopic properties of the microbial surfaces.<sup>7</sup> These studies have provided an integrated insight into the factors that govern attachment to the oil-water interface, and the behavior of the oil-water interface as a result of the presence of the bacteria. Further studies are examining the impact of interfacial films of bacteria on the migration of droplets through porous media, as in aquifers and oil reservoirs, and defining the distance of the cell wall from the actual oil-water interface. These nanometer distances determine the potential transport mechanisms for cellular uptake of hydrophobic compounds at the interface.

At the University of Waterloo, Bill Anderson, Marc Aucoin, Perry Chou, Eric Jervis, Ray Legge, Murray Moo-Young, Christine Moresoli, and Jenő Scharer are engaged in bioengineering research. Investigations are underway on mixed microbial communities with applications in biofiltration and constructed wetlands. Ecoplates and principal component analysis of large data sets are used in this work as part of the development of integrated or semipassive treat-

ment systems for wastewater mitigation or pathogen removal, including wetlands and small-scale treatment systems. In addition, fat and oil transformations with emphasis on the development of multienzyme transformations are being studied, including the development of novel enzyme supports. Other applications of this work include the development of feedstock materials that may be suitable as a petroleum replacement in biobased materials for the automotive sector or in the production of clean alternative fuels.

Genette Turcotte's group at Ryerson University is working on unit operations required to produce ethanol from lignocellulosic agricultural residues including pretreatment steps, enzymatic degradation, simultaneous fermentation of the hexoses and pentoses, and pervaporation.<sup>8</sup> The group has evaluated animal nutrition from the partly degraded solid residues, and has successfully produced one of the expensive microbial cellulases from tobacco chloroplasts using molecular biology techniques. Turcotte is currently working on mixing problems associated with high-density suspensions of these residues, evaluating the synergistic action of purified microbial cellulases to accomplish the degradation, and immobilizing the enzymes to measure their binding strength on plant fibers. She is also collaborating on simultaneously producing other enzymes required for complete degradation of the residues. Also at Ryerson, Mehrab Mehrvar's main research focus is on the design and optimization of integrated advanced oxidation technologies and biological processes for the treatment of drinking water and wastewater.<sup>9</sup> This integration confers higher efficiency of organic pollutants removal, while simultaneously reducing the total time and cost of the processes. Advanced oxidation processes are used in pretreatment of the nonbiodegradable and refractory contaminants followed by the biological processes.

At the University of Saskatchewan, Gordon Hill has been developing a recombinant yeast strain harboring barley amylase expressing genes, which can utilize starch as the starting material during ethanol fermentation. As a result, a more efficient ethanol production can be realized. Hill also develops various biological processes to propagate green algae and other microorganisms to sequester atmospheric carbon, to remediate air-borne volatile organic compounds from petroleum operations, and to degrade water- and soil-based carcinogenic chemicals such as naphthenic acids, one of the end products found in the tailing stream of the oil-sand extraction process. Mehdi Nemati's research at Saskatchewan targets environmental problems associated with petroleum and mining industries. These include the biotreatment and management of specific waste streams such as acid mine drainage, sour gases, sulfide and nitrate laden waters, and in situ control of biogenic H<sub>2</sub>S production and the associated corrosion in oil reservoirs. The possibility of generating energy during the biotreatment of H<sub>2</sub>S and nitrate containing streams in microbial fuel cell type bioreactors is also being investigated. Nemati is also involved in research aiming to develop a technology for bioremediation of soils and waters contaminated with polycyclic aromatic hydrocarbons and naphthenic acids, a major environmental issue associated with the processing of oil sands in Western Canada. Yen-Han Lin combines biochemical engineering, molecular biology, and bioinformatics to study the physiology of *Saccharomyces cerevisiae* during very high gravity fermentation. He utilizes mass spectrometry and microarray technology to explore yeast genomic, proteomic, and metabolomic profiles

under industrial ethanol production conditions. He also develops various bioinformatics tools to integrate the vast amount of data collected from those experiments, and to extract and analyze embedded biological information, toward a better understanding of yeast functionality.

At UBC, Madjid Mohseni's research is focused on the bioproduction of hydrogen from cellulose-based organic wastes. The use of waste biomass as a feedstock for H<sub>2</sub> production is CO<sub>2</sub> neutral, and Mohseni, in collaboration with Charles Haynes, Sheldon Duff, and Susan Baldwin, is applying anaerobic biological fermentation to produce hydrogen from pulp and paper waste materials.

At the University of Ottawa, Jules Thibault has three areas of research underway. One project deals with the production of pullulan, a biopolymer that is produced by *Aureobasidium pullulans*. This biopolymer is water-soluble and edible and has numerous applications, but the one that the Thibault group is focusing on is its use for scaffolding material in biomedical applications. A second project deals with the production of cellulase used in the saccharification of lignocellulosic material in view of producing ethanol. Using *Trichoderma reesei*, Thibault is trying to determine the intimate relationship that exists between the morphology, enzyme production, and operating conditions with the objective to optimize the production of cellulase. The third current project deals with the production of ethanol and its recovery from fermentation broth. Also at Ottawa, Christopher Lan is focusing on microalgae that have the highest growth rate and diversity among photosynthetic organisms and a promising species for the production of biofuels. Lan's objective is to develop advanced technologies for the cost effective production of biofuels using fast growing microalgal species under Canadian climate conditions for the combined benefits of renewable energy production, pollution control, and CO<sub>2</sub> mitigation.

In Montreal at the Biotechnology Research Institute of the National Research Council of Canada, Peter Lau is making use of microbes as environmental and sustainability tools to access novel platform chemicals, create new value addition and bioproducts from feedstocks such as natural fibers or biomass in a biorefinery setting. Through genome mining and directed evolution technique, advanced biocatalytic systems (e.g., pectinases, cutinase) are being developed to process bast fibers such as hemp and flax for their use in biocomposites for the textile industry. He is also interested in the production of enantiomerically pure chiral compounds through a wider use of biocatalysts such as Baeyer-Villiger monooxygenases.<sup>10</sup> Since many value-added chemicals are water-insoluble, and often cellular toxicity is a problem in bioprocessing, variations of the two-phase partitioning bioreactor (TPPB) system are being designed to optimize biocatalyst longevity and product recovery. As a whole, green chemistry, through less polluting and more efficient bioprocesses, is sought to address the ever-increasing issues of environmental protection and industrial sustainability.

At the University of Sherbrooke, biological processes and biotechnology are used in environmental research to treat air, water, and industrial wastes. For example, Michèle Heitz and Peter Jones use biofilms to treat VOCs. Jones has developed methods to treat water and remove endocrine disrupter chemicals using free and immobilized laccases. Researchers in the department are also working on the elimination of industrial wastes such as pig manure or whey with a view of producing valuable products. Esteban Chornet is undertaking

research on the production of ethanol from cellulose and plans to construct two demonstration plants for the production of ethanol. Researchers at Sherbrooke have also undertaken projects to produce biodiesel from a number of starting materials.

At Queen's University, Juliana Ramsay is working on dye decoloration using fungal culture to treat colored effluents. In addition, naphthalene, dioxane, biphenyl, and ethylene glycol in contaminated sediments are being biodegraded. Together with Bruce Ramsay, she works on high cell concentration fermentations (80 g/L) using forest and agricultural waste as feedstock. Andrew Daugulis at Queen's is applying the TPPB concept for the biodestruction of toxic organic compounds (xenobiotics). In this scheme, the presence of an inert and immiscible second phase acts as a reservoir for extremely high loadings of toxic substrates which partition to the cells in the bioreactor aqueous phase based on the system maintaining thermodynamic equilibrium and the metabolic demand of the cells.<sup>11</sup> Xenobiotics such as BTX compounds (benzene, toluene, xylene), as well as chlorinated and polyaromatic compounds present as solids, liquids, or in gas streams, can be degraded to completion at unprecedented rates in these self-regulating systems. Although biocompatible organic solvents continue to be used as the substrate delivery phase, the Daugulis group has recently shown that solid polymer beads, in the form of plastic pellets, can act in an identical fashion in the uptake and release of toxic molecules, maintaining them below inhibitory levels within TPPBs.<sup>12</sup> Since the polymers are completely nonbiodegradable and biocompatible, the Daugulis group has been able to exploit the use of widely mixed cultures of organisms, inherently superior to pure strains because of their metabolic diversity, in these challenging bioremediation applications. Also at Queen's, Caroline Baillie is working on the use of natural fiber composites as environmental materials for applications such as low-cost housing. Pascale Champagne, cross appointed from the Civil Engineering Department at Queen's, works on the development of environmentally sustainable water and waste management strategies. Of particular interest is the extraction of value-added products from municipal, industrial, and agricultural wastes, and the development of management strategies and passive treatment systems for various wastewaters. Also in civil engineering, Bruce Anderson is developing plant- and bacterial-based wastewater and landfill leachate treatment systems for small urban and rural developments. Demonstration installations are located in Kingston, Bayside, and Portland, ON, and future work will take place in China. A zero-discharge plant-based treatment system (Ecocyclet<sup>TM</sup>) has been developed using wastewater as a resource for biomass growth.

## Fermentation and Bioprocessing

At the University of Waterloo, research is focused on developing innovative strategies to improve microbial strain ability for biomanufacturing via physiological manipulation, in particular to enhance the production of recombinant proteins and biofuel metabolites. Several heat-shock proteins with protease or chaperone activities have been manipulated for improving *in vivo* protein misfolding during the course of gene overexpression. Other work has focused on novel behavior in very high gravity ethanol fermentations. Research on optimization of recombinant protein production

in various cell platforms has included recombinant human interleukin 3 production by *Streptomyces lividans*, production of amylase by fed-batch culture of *Bacillus subtilis*, and recombinant protein production by *Aspergillus niger*.

Jason Zhang at the University of Ottawa is investigating the applications of biochemical engineering principles in the mass production of bioproducts and in the reduction and/or elimination of adverse environmental impacts from industrial activities. Currently active projects include *Pichia pastoris* fermentation for the mass production of recombinant phytase and photobioreactor development for full-scale efficient microalgae cultivation. Also at Ottawa, Christopher Lan is currently working on the development of high-efficiency food-grade platforms based on wild type or recombinant lactic acid bacteria (LAB) for the production of novel bioproducts, biofuels from microalgae, and novel alternatives to antibiotics.<sup>13</sup> LAB are a well-studied and GRAS group of industrially important bacterial species making them an appropriate food-grade platform for the production of bioproducts such as live oral vaccines. Lan's studies in high-cell density cultivation of LAB aim to improve fermentation performance, and future research is intended to be directed to the development of oral vaccines using recombinant LAB species. Lan's group is also developing technologies for the propagation and purification of phages of interests to phage therapies and identifying virolysins, a type of highly specific and efficient phage-encoded lytic enzymes, as novel alternatives to antibiotics.

At the University of Sherbrooke, a research priority is to design, develop, and control bioprocesses allowing the production of specific biomolecules using several cell culture systems (bacteria, fungi, mammalian, and plant cells). Joël Sirois, with a background in industrial bioprocesses, particularly in the cultivation of plant cells to produce biomolecules, is employing a systems biology approach to describe the dynamic behavior of bioproduction systems. Nicolas Abatzoglou is undertaking studies in pharmaceutical engineering and is developing particulate systems which are used to produce medications in the form of tablets and capsules.

At Queen's University, Bruce Ramsay is producing poly-3-hydroxyalkanoates through fermentation process and is also developing high cell concentration fermentations. Andrew Daugulis has applied the concept of TPPBs for the enhanced bioproduction of high-value pharmaceutical intermediates by in situ product removal (ISPR) techniques. The two phases in these systems consist of a cell-containing aqueous phase in which the synthesis of high-value molecules occurs, and a second immiscible phase whose sole purpose is to selectively sequester toxic target products away from the cells. The TPPB technology platform has been successfully applied to the production of inhibitory fermentation products such as ethanol and butanol, as well as the production of 3-methylcatechol, an intermediate in the manufacture of the antivasular cancer drug combretastatin A-1.<sup>14</sup> The Daugulis group has recently demonstrated that solid polymer thermoplastic beads can act in an identical fashion to immiscible organic solvents in the uptake and release of toxic molecules, maintaining them below inhibitory levels within TPPBs. The use of inexpensive, nonvolatile, nonflammable, biocompatible, and easily shaped polymers as the sequestering phase in a bioreactor is a step toward the design of high-efficiency, low-cost bioprocesses that eliminate cell toxicity and the development of "green," solvent-free processing strategies.

## Control and Modeling

Olivier Henry at Ecole Polytechnique is currently developing control strategies for mammalian cell culture processes. The complex behavior of mammalian cells in culture is currently only partially understood because of the multitude of parameters governing cell growth, metabolism, and productivity. In turn, this lack of knowledge has hindered the development of effective control strategies, which are vital for reducing production costs, ensuring high yields and maintaining the product quality. Henry's research focuses on the development of accurate metabolic models providing a quantitative description of the physiological state of mammalian cells in culture as a tool for process optimization. The approach relies on isotopic tracer studies combined with mass spectrometry analysis and mathematical modeling. These research activities are targeted toward the improvement of both recombinant protein and viral vector production processes. Michel Perrier has been developing fed-batch processing strategies. His research focus is on the design of high-performance control and optimization algorithms that can cope with the fact that kinetic models are generally inaccurate and that there is a lack of adequate on-line sensors for key variables. Mario Jolicoeur also at Ecole Polytechnique is undertaking the development of noninvasive on-line monitoring tools to improve observations of cell physiological states and their behavior in bioreactors. A small-scale bioreactor of 2–3 mL for in vivo NMR has been developed for plant and mammalian cells. <sup>31</sup>Pi NMR allowed monitoring plant cells metabolic (pH, Pi, G6P, F6P) and energetic states (ATP, ADP), including cell compartments. In parallel, an innovative and automated method for the on-line monitoring of secreted protein concentration and bioactivity is being developed, by harnessing a surface plasmon resonance-based biosensor to a bioreactor. These monitoring tools are applied to diverse systems such as recombinant production bioprocesses and metabolic diseases. In other research, Jolicoeur is developing a kinetic-metabolic model capable of describing cell potential to grow and produce a molecule of interest. First developed for plant cells, a kinetic-metabolic structured model has been developed based on key intracellular nutrients accumulation (Pi, nitrogenous compounds, and carbohydrates). The model is now applied at developing efficient medium feeding and control strategy to manipulate the cell nutritional state and enhance the production potential in highly valuable pharmaceuticals such as secondary metabolites and recombinant proteins of pharmaceutical interest. He is now developing such dynamic-metabolic model for mammalian cells.

At the University of Ottawa, David Taylor is modeling bioartificial organs. Thousands of people die each year as a result of organ failures of various kinds. Although transplants offer some measure of hope for patients facing disorders such as fulminant liver failure, a shortage of viable donor organs and the resultant delays lead to premature death. One possible short-term solution is to place these individuals on bioartificial organs containing viable cells that carry out the vital functions of the particular organ until a permanent solution is found. Taylor's research group has developed models of such bioartificial organs to gain insights into their proper application in clinical settings. In related work, Taylor is modeling mass exchange in body tissues which is vital for maintaining fluid balance within the body. Mathematical models of the microvascular exchange system developed by Taylor are being applied to better understand

the processes of fluid and solute exchange within the body, and so to seek improved clinical treatments. The utility of these models extends beyond issues of fluid balance and can readily be applied to the study of various drug delivery schemes.

At the University of Waterloo, research using high-resolution imaging and photonics-based analytical techniques that incorporate systems biology and informatics to gain insights into cellular dynamics and epigenetic trajectory has been applied to image-based lineage tracking tools for examining cell division symmetry and cell potential maintenance in stem cell cultures. A generic methodology including metabolic flux analysis (MFA), partial least squares analysis, and dynamic modeling have also been developed for the control and optimization of hybridoma culture. Through nutritional profiling, using principal component and PLS methods, the medium components were ranked for their effect on MAB productivity, cellular growth, glutamine and glucose consumption with the aim of optimizing MAB production via fed-batch operations.

Mehrab Mehrvar at Ryerson University has an interest in optimizing feeding strategies in microbial batch and fed-batch fermentations to increase the cell mass density, and therefore by extension, possibly improve production yields.

At the University of Toronto, Ken Norwich has been developing a comprehensive model governing the regulation of blood glucose that incorporated the role of incretins, a newly discovered hormone.<sup>15</sup> Krishna Mahadevan also models biological systems with a focus on metabolic and gene regulatory networks.<sup>16</sup> His laboratory uses bioinformatics, computational, and systems biology methods to reconstruct metabolic pathways and gene regulatory networks with practical applications in health, environment, and bioenergy. Thus Mahadevan straddles both biomedical and bioenvironmental camps, and he was the first to develop a mathematical model of metabolism of an anerobe, *Geobacter sulfurreducens*, an important metal reducing organism with applications in bioremediation and bioelectricity generation.<sup>17</sup>

### Biomaterials and Molecular Delivery Systems

Hasan Uludag's lab at the University of Alberta is concentrating on the design of novel carriers for protein and DNA based therapeutics. Carriers are synthesized and engineered at the molecular scale that accommodate unique properties of the chosen therapeutic agents as well as the delivery challenges posed in a physiological milieu. Bone-specific delivery methods are being designed for proteins by formulating bone-seeking bisphosphonates with bone-stimulating growth factors.<sup>18</sup> Novel bisphosphonates are being engineered for maximizing bone delivery while reducing nonspecific distribution of therapeutic agents to other anatomical sites. Cationic polymers are engineered for DNA delivery by incorporating lipids into the polymers, ultimately for genetic modification of clinically relevant cells.<sup>19</sup> By using biomimetic mechanisms, the Uludag lab aims to control the intracellular fate of DNA therapeutics with engineered carriers.

Research at McMaster University on materials for use in blood contacting devices has been ongoing for many years with focus on devices such as hemodialysis, heart-lung bypass, prosthetic heart valves, vascular stents, arterial grafts, and catheters. John Brash, Heather Sheardown, and

Shiping Zhu are conducting research along two main lines: mechanistic studies of the interactions of blood with "artificial" surfaces and materials development per se. The emphasis in the mechanistic studies is on protein interfacial behavior, with a major theme in this work being the unraveling of the "proteome" of protein adsorption, i.e. determination of the composition of the multicomponent protein layer that forms within seconds at the blood-material interface utilizing a variety of methods including radiolabeling, immunodetection and, most recently, modern proteomics approaches. On the materials development front, information from the mechanism studies has been exploited; for example, protein resistant surfaces having a variety of structures have been developed using polyethylene oxide and more recently phosphorylcholine (PC)-containing polymers. A recent achievement is the development of highly protein-resistant surfaces based on 2-methacryloyloxyethyl phosphorylcholine (MPC). These were prepared by surface grafting using atom transfer radical polymerization (ATRP) methods.<sup>20</sup> Recently, initiated work with these materials involves conjugation of MPC polymers to therapeutic proteins, a process similar to PEGylation. The purpose is to extend circulating time and to improve the efficacy of the drugs. The MPC-protein conjugates are expected to have improved stability in many biomedical applications. A second example in the cardiovascular area is based on the concept of a material that will actively dissolve clots which begin to form upon blood contact. The surface is designed such that it will adsorb preferentially from blood the proteins (plasminogen and tissue plasminogen activator) that are required for clot lysis. Also at McMaster, Heather Sheardown and Mike Brook are pursuing work on ophthalmic biomaterials. The focus is on the development of novel polymeric biomaterials for delivery of drugs as well as on understanding and manipulating the interactions of these materials and surfaces with the surrounding cells and proteins. Examples include development of artificial corneas and corneal onlays for the restoration of sight, drug delivery to the eye, and modification of intraocular lenses for reduction of posterior capsule opacification. In the cornea work, two approaches are being followed: modified polymers for scaffold materials that are designed to promote interactions with corneal cells, and a tissue engineered cornea based on collagen, the structural component of the normal cornea.<sup>21</sup> The drug delivery work is concerned mainly with delivery to the back of the eye for the treatment of diseases such as age-related macular degeneration and diabetic retinopathy. One project is exploring the use of alginate microspheres for the release of protein drugs; another uses a MEMS-based system for targeted and controlled drug release. The work is focused on using the signaling molecules involved in PCO formation in conjunction with lens materials to develop novel intraocular lenses, which show lower amounts of PCO. Bob Pelton, also at McMaster, is directing a network dedicated to develop bioactive papers that will detect, capture, and deactivate pathogens. The idea is to incorporate biologically active molecules (antibodies, aptamers, enzymes, etc) into the paper. A broad range of applications is anticipated including testing for and removing pathogens from water, detection of pathogens on surfaces (e.g. surfaces used for food preparation), and low-cost paper-based diagnostics.

Michael Buschmann's research at Ecole Polytechnique focuses on three areas: chitosan biomaterials, cartilage and meniscus repair, and gene delivery. Buschmann has developed particular formulations of chitosan that can be used in

conjunction with blood, cells, and other biological components in order to repair tissues and have other therapeutic effects. Fundamental research involves the study of the polyelectrolyte behavior of chitosan in solution and in particular its interesting thermal gelation upon heating. The molecular mechanisms involved in gelation and other physicochemical phenomena are being investigated both experimentally and with theoretical mathematical models of polyelectrolytes in solution. Research efforts also involve development of processes to control molecular weight and acetylation and the development of analytical methods to precisely characterize these two parameters. In the area of cartilage and meniscus repair, the Buschmann lab is studying the structure, biomechanics, and biology of articular cartilage and meniscus, and using chitosan-based biomaterials to develop means of regenerating these tissues when they are damaged. His approach relies on formulating mixtures of chitosan solutions and blood components that can be applied to cartilage and meniscus lesions and stimulate their repair by activating host cell recruitment, vascularization, and synthesis of an effective repair tissue. Success has been achieved in improving the repair of articular cartilage in animal models, and this technology has been translated to industry for clinical application. Buschmann's lab also uses chitosan as a carrier to take DNA into a cell for gene delivery purposes to stimulate tissue repair or to have therapeutic effects in cardiovascular, cancer, and metabolic disease. They have created chitosan/DNA nanoparticles that achieve very high levels of transgene expression and are studying the mechanisms responsible for these high transfection levels using both physicochemical characterization of chitosan-DNA interactions and the cell biological events involved in the transfection process. In the area of biomechanics they have been working on new instruments that test the biomechanical properties of cartilage when it is compressed and mathematical models to predict the forces developed in cartilage under different loading conditions. This has led to technology that has been translated to industry in the design of an instrument that is being used to map the function of articular cartilage in patients undergoing arthroscopic surgery. Gregory De Crescenzo also at Ecole Polytechnique undertakes research in the area of biomaterials and regenerative medicine and is focusing on the in-depth biophysical characterization of macromolecular interactions, i.e. receptor-growth factor and protein-biomaterials interactions. The ultimate goal of this research is to develop innovative molecular strategies to tether growth factors in an oriented and optimal fashion for the development of a new generation of biomaterials: bioactive materials for controlled tissue repair and regeneration. Caroline Hoemann is engaged in a multidisciplinary effort that combines biopolymers, biophysical models, in vitro cell culture assays, imaging, biochemical analyses, mechanical testing, animal models and fine histological methods to research fundamental mechanisms of bone and articular cartilage regeneration, and develop new medical devices for detection and treatment of damaged cartilage, particularly in the knee. Hoemann's approach to repair damaged cartilage uses in situ solidifying scaffolds to stimulate and modify normal repair processes. Her research is also concentrated on using biodegradable scaffolds for targeted delivery of genes and gene products to repairing wounds. A medical device to treat focal articular lesions, BST-CarGel (TM), has been developed, licensed to industry, and is currently in clinical trials.

At the University of Waterloo, Pu Chen's work on nanomaterials is elucidating a mechanistic understanding of nano-

fiber growth of ionic-complementary peptides on surfaces.<sup>22</sup> The growth mechanism revealed two steps: adsorption of nanofibers and fiber clusters from the bulk solution on the surface ("seeding"), and fiber elongation of the "seeds" from their active ends. The results provide insights into the assembly of peptide/protein on a surface, essential to understand such physiological protein aggregation systems as amyloid fibrillogenesis. In parallel, Chen's group is elucidating the role(s) of copper salts containing different anions on the self-assembly of a designed peptide with affinity for copper ions.<sup>23</sup> The results have contributed to an understanding of self-assembly and nanowire fabrication using biomolecules, self-assembling peptides in this case. Such complementary peptides have formed the foundation of carriers for hydrophobic drugs.<sup>24</sup>

At the University of Ottawa, Xudoing Cao's research is focused on biomaterials and tissue engineering with special interests in regenerating damaged tissues for repairs. Two general directions are being pursued: studying the influence of material-cell interactions as well as cell-cell interactions on mammalian cell proliferation and differentiation via microfabrication techniques and preparing a novel bioinspired bioengineering scaffold for tissue regeneration. Biodegradable scaffolds with well-controlled porosity are created in the laboratory to mimic the structures and the mechanical properties of those of the targeted organs in the body, for example, bones. To achieve this, supercritical CO<sub>2</sub> foaming technique is used. Cao anticipates that the resulting scaffold has better (and tuneable) mechanical properties for a given materials mass used to prepare the scaffold in comparison with traditional scaffolds. In addition, by combining microfluidic flows, microfabrication, and laser detection, a high-throughput and inexpensive pathogenic bacteria detection system is being developed in the Cao group. This technology has the potential to detect food-borne pathogens in the food chain and let authorities screen for suspicious food or drinking water contaminations in the event of terrorist attack on food supplies.

The bioengineering research in Wankei Wan's lab at the University of Western Ontario is concentrated on the design, fabrication, modification, and characterization of novel nanobiomaterials and their composites for biomedical and medical device applications. Nanobiomaterials investigated include bacterial cellulose<sup>25</sup> and electrospun nanofibers of proteins and polymers<sup>26</sup> for controlled release/drug delivery and medical device applications related to wound healing and cardiovascular purposes.<sup>27</sup> Another area of research is in tissue regeneration and tissue engineering using mathematical modeling and simulation to evaluate and optimize medical device design and tissue bioreactor design.

Yaser Dahman at Ryerson University is undertaking research on the design, fabrication, and utilization of novel biocomposites.<sup>28</sup> These biocomposites range from simple blends to crosslinked polymers, interpenetrating polymer networks, grafted polymers as well as copolymers. Special interest is devoted toward the bacterial cellulose-based biocomposites, in which biocellulose nanofibers are used as a reinforcing element. Synthesized in static and agitated cultures, bacterial cellulose is a natural nanomaterial that has a native dimension of less than 50 nm in diameter with unique physical properties that distinguish it from plant-derived cellulose. Because of possible modifications of the cellulose nanofibers, current and potential applications range from the commercially produced plastic celluloid, to contact lenses,

drug delivery systems, cellular therapies (scaffolds), biosensors, and nanomembranes.

At the University of Toronto, an extensive research effort in the area of Biomaterials and Molecule Delivery Systems has been underway for many years. Michael Sefton has made significant contributions in the areas of cell encapsulation and blood-material interactions. For cell encapsulation, Sefton is particularly interested in modulating the immune and inflammatory response using genetically modified cells to these capsules upon implantation. His laboratory has also demonstrated that so-called immunoisolatory capsules, in which cells were suspended, allowed inflammatory factors to diffuse through their protective membranes, thereby causing an immune response.<sup>29</sup> In addition to advancing understanding of biomaterial-associated inflammation, Sefton is focusing on one of the key limitations in tissue engineering research—vascularization of scaffolds to ensure cell viability deep within. This has resulted in the creation of novel modular scaffolds for tissue engineering.<sup>30</sup> Using biomaterials to influence cell fate is being actively pursued by Molly Shoichet's laboratory at Toronto. Three-dimensional scaffold design has been developed by Shoichet's laboratory, where biomimetic, chemically patterned scaffolds were used to guide cell growth with either cell-adhesive ligands or immobilized growth factor concentration gradients.<sup>31</sup> Shoichet's biomaterials have been advanced in drug delivery applications for both spinal cord injury repair, where some therapeutic benefit was observed with the biomaterial itself,<sup>32</sup> and cancer, where biodegradable polymers self-assemble into nanoparticles that have been modified with antibodies for targeted delivery. Yu-Ling Cheng's research in drug delivery and biomaterials is focused on microfluidics, and Edgar Acosta's research with formulation chemistry, surfactants, and emulsions is being tested in drug delivery and food additives, among others. Craig Simmons is focusing on cell-matrix interactions and cellular response to biochemical (e.g., growth factors) and physical (e.g., flow-induced shear stress) stimuli. Simmons' group recently discovered that heart valve endothelial cells exhibit unique morphological and adhesive properties from vascular endothelial cells when grown on different extracellular matrix proteins using microfluidics.<sup>33</sup>

At UBC, the central objective of Charles Haynes' research program is to improve understanding of the interfacial behavior of biomolecules and cellular systems, making it possible to build instruments capable of controlling such interactions. Haynes is exploring the molecular basis of such interactions, and then applying this fundamental knowledge to the design of natural or synthetic surfaces that may make it possible to analyze complex biosystems at the molecular level. Donald Brunette's laboratory at UBC was among the first to apply techniques developed for the production of microelectronics to produce precisely controlled surfaces to study cell behavior.<sup>34</sup> Procedures were developed to replicate etched silicon surfaces in epoxy, coat them with titanium, and process implants for light and electron microscopy. The mechanism of contact guidance on microfabricated surfaces was extensively studied as has been the role of the cytoskeleton in cells exhibiting contact guidance.<sup>35</sup> Recent studies include the effects of topography on cell signaling<sup>36</sup> and studies on the effects of microfabricated surfaces on cell behavior *in vivo*. This work has demonstrated a wide range of effects of surfaces, and provided evidence that contact guidance, an *in vitro* phenomenon, occurs on implants *in vivo*.

A number of researchers at the University of Sherbrooke (Esteban Chornet, Nathalie Fauchoux, Patrick Vermette) are undertaking research on delivery systems to control the release of biomolecules using different technologies such as diffusion control or chemical reaction control. Fauchoux's research group is working on the intracellular biochemical events induced by the cell-biomaterial interactions. Such events regulate subsequent cell behaviors such as cell survival, proliferation, and differentiation, and a better understanding of these cell-biomaterial interactions is required to develop new biomaterials such as biomimetic materials to favor tissue repair or to create specific bone substitutes. Research in material sciences at Sherbrooke has also been focused on the development of new materials including nanomaterials, polymers with specific surface properties that are important in the biotechnological fields including bioreactor design and tissue engineering. François Gitzhofer has developed plasma systems to produce coatings and ultrafine powders for chemical and ceramic synthesis. Ceramics with specific composition, porosity, and interconnection are currently under development to create innovative bone substitutes. Patrick Vermette and Pierre Proulx are developing a platform for surface science and tissue engineering studies. One of their objectives is to promote new technologies and biotechnological processes to allow large-scale production of vascularized human tissue.

At Queen's University, research in Brian Amsden's group involves the design and preparation of degradable polymer biomaterials. Current work includes the preparation of photocrosslinkable biodegradable elastomers, photocrosslinkable polysaccharides, and low-viscosity biodegradable thermoplastics. These polymers are being designed for use as growth factor delivery vehicles, as surgical materials, and as scaffolds for the growth of new soft tissues such as ligaments and cartilage. Characterization of these materials ranges from spectroscopy techniques to determine structure and composition, thermal techniques, and animal implantation studies to determine host-tissue response and biodegradation rates. Also at Queen's, Ron Neufeld is using natural and chemically modified polymers to encapsulate peptide-based drugs. Three formulation methodologies have been developed to produce nanoparticles containing insulin as example. The nanocomplex consists of a polyuronic acid-dextran core containing insulin, coated with chitosan-polyethylene glycol-albumin, and when delivered by oral administration or subcutaneous injection to diabetic rats has shown high pharmacological availability. Long-term toxicological studies are presently being undertaken on the orally dosed diabetic rats. Neufeld is also working on the microencapsulation of biological materials including living cells, enzymes, and bioactive materials. A recent project involves the development of a granulation process for subtilisin, an industrial enzyme produced commercially on large scale for use in detergent powders. Through this work, a "technology platform" has been developed involving the large-scale production of size controlled and environmentally responsive granular beads, which are subsequently loaded with active enzyme or bioproduct using a method termed absorptive granulation. Kim McAuley is working to develop improved polymer gel dosimeter recipes and read-out techniques that result in more accurate, reliable, and cost-effective measurements of radiation dose distributions. Polymer gel dosimetry is a promising technique for verifying spatial radiation-dose distributions delivered by cancer radiotherapy equipment. To

check whether the radiotherapy equipment is delivering the correct radiation dose to the correct location, medical physicists irradiate a small polymerization vessel (the phantom) in place of the patient. The phantom contains a mixture of water, gelatin, a monomer (e.g., *N*-isopropylacrylamide), and a crosslinker (e.g., *N,N'*-methylene-bisacrylamide). The radiation induces free radical copolymerization and crosslinking reactions, producing polymer microgels that precipitate from the solution. The amount of polymer produced at each location depends on the locally absorbed radiation dose determined by three-dimensional radiation dose maps.

Diego Mantovani's research team at Laval University is focusing efforts on improving the performance of biomaterials and commercial devices by designing and validating new materials and structures as substrates for the scaffolding and regeneration of cardiovascular tissue. Work on the permanent grafting of molecules on commercial arterial prostheses to stimulate the proliferation and growth of endothelial cells<sup>37</sup> is underway as is the stable grafting of bioactive molecules onto the surface of metallic medical devices by chemical etching and electropolishing. This is intended to improve the adhesion of the polymer directly onto the metal rather than the oxide layer, which is chemically unstable and mechanically brittle and therefore cracks when the stent is deployed.<sup>38</sup> Structures and processes for scaffold-based vascular tissue engineering, from both natural and synthetic polymers,<sup>39</sup> are being developed with particular focus on the effects of different treatments (including UV) on enhancing the structure/properties relationships of these scaffolds. Advanced alloys for the development of a new class of metallic medical devices are under development.<sup>40</sup>

### Tissue and Cell Culture Engineering

At the University of Toronto, Milica Radisic complements Mike Sefton's work on scaffolds, where channeled 3D scaffolds, perfusion bioreactors, and coculture of cardiac myocytes and nonmyocytes are advanced for vascularization of cardiac tissue engineered scaffolds.<sup>41</sup> Building on the previous findings that electrical field stimulation improves functional properties of engineered cardiac tissue, she also uses microfabricated systems to investigate the interactive effects of multiple physical stimuli (e.g. contact guidance and electrical stimulation) on the phenotype of heart cells.<sup>42</sup> Peter Zandstra and Julie Audet at Toronto are examining bioreactor design for stem cell bioengineering while advancing understanding of stem cell biology. Research in the Zandstra Laboratory is focused on the generation of functional tissue from adult and embryonic stem cells. His quantitative, technology-driven approach strives to gain new insight into the fundamental mechanisms that control stem cell fate and to develop robust systems for the controlled development of stem cells and their derivatives. Recent results from the Zandstra lab have demonstrated that embryonic stem cells form *in vitro* autoregulatory niches<sup>43</sup> and that interactions between stimulatory and inhibitory signaling networks balance self-renewal and differentiation as a function of niche properties such as size and composition.<sup>44</sup> Research in the Audet laboratory is focused on cellular and molecular mechanisms responsible for apparent cytokine synergism in stem cell cultures, development of cell-permeable peptide constructs, and single cell electrophoresis. Understanding molecular self-assembly and specifically protein-ligand and biomolecular complexes is the focus of Chris Yip's research

program at Toronto, where he has demonstrated protein complexes at interfaces using custom-designed scanning probe microscopy. Warren Chan's group is also actively engaged in engineering new instrumentation and techniques to address biological questions, such as the proteomic and genomic changes associated with abnormal cells (e.g., cancer cells or virally infected cells) and tissues. Chan aims to elucidate the cell's molecular dynamics by using nanotechnology, microtechnology, and molecular engineering.

At McMaster, fundamental work on tissue engineering is the main interest of Kim Jones. The ultimate goals of this work are to understand the molecular processes involved in the host response to biomaterials and to exploit that knowledge in designing novel biomaterials and systems for purposes from tissue engineering to vaccine design.

One project is aimed at understanding the causes and consequences of the inflammatory response caused by polymeric biomaterials. Recent results suggest that biomaterials are sensed through the innate immune system. It has been found that soluble alginate causes nuclear translocation of NF- $\kappa$ B in macrophage cells, in addition to stimulating secretion of a range of cytokines, much as endotoxin does. In contrast, solid alginate microspheres appear to suppress such responses. These findings suggest that solid alginate is an appropriate material for use in tissue engineered constructs, whereas alginate solution may be useful as a vaccine adjuvant. The interaction between inflammation and fibrosis is also under investigation. Preliminary work suggests that biomaterials-induced fibrosis is not correlated to the extent of inflammation. Indeed, inflammation can be stimulated with no corresponding increase in fibrosis. A recent line of investigation involves the design of cells for use in tissue engineering by mimicking the methods used by viruses to evade immune detection in genetically engineering allogeneic cells.

James Piret at UBC is undertaking the engineering of mammalian cell processes in the Michael Smith Laboratories. Piret's group is primarily focused on mammalian cell processes for either therapeutic protein production or cellular therapy. This multidisciplinary research ranges from molecular biology to bioreactor engineering and often includes collaborative work with basic scientists. In collaboration with StemCell Technologies (Vancouver, BC) and a national network of stem cell researchers, the group investigates the critical factors that influence stem cell production, gene therapy efficiency, and the differentiation of islet cells for diabetes therapy. In the biotechnology area of recombinant protein production, Piret's group investigates methods to accelerate the R&D of production processes. The lab also works on devices such as a commercialized acoustic filter developed in Piret's lab. Sue Baldwin at UBC is interested in developing technologies for women's healthcare. One aspect of Baldwin's research concerns the use of minimally invasive technologies in women's health care. For example, in collaboration with industry partners, her students assessed the safety and efficacy of a thermal ablation treatment for menorrhagia through mathematical and experimental models. Current projects include studying tissue implants for permanent pregnancy prevention, which are alternatives to tubal ligation, and implants for prevention of uterine prolapse, a common problem faced by the elderly. Baldwin's research focuses on the interaction between the device or implant and the surrounding tissue, using animal models and mathematical modeling so as to examine the safety and efficacy of the procedure. Baldwin's research group is also studying

biomarkers in cancer using blue mussels, *Mytilus sp.* Although these organisms are used as sentinels for ecological health, the cancer and associated pathway that are being monitored have many similarities to that in humans. Researchers in the laboratory are using quantitative polymerase chain reaction and mutation analysis as techniques for determining the p53 family and other genes in the pathway as biomarkers for leukemia. Already, p53 and several of its isoforms have been discovered and sequenced in *Mytilus trossulus* and *M. edulis* and interesting p53 mutations identified in Baldwin's laboratory.

At the University of Calgary, the Cellular and Molecular Bioengineering Research Laboratory (CMBRL) is run by Kristina Rinker. CMBRL research efforts are focused upon combining engineering principles with cell biology, molecular biology, and biochemistry in the investigation of the effects of physical and biochemical forces on human cell physiology, particularly in the area of vascular health and disease mediated by endothelial cells.<sup>45</sup> The presence of endothelial cells at the interface of blood flow and the arterial lumen suggests that many factors affecting arterial health are likely to be mediated by the endothelial layer. Current studies focus on the effect of shear stress magnitude on signal transduction pathways and gene transcription in endothelial cells preconditioned to prolonged fluid shear stress. Cardiovascular disease has been shown to be an inflammation-dependent disorder initiated by endothelial upregulation of white blood cell adhesion receptors and propagated by generation of inflammatory biochemicals (cytokines) and adhesion of white blood cells. Endothelial adhesion receptor expression is altered by spatial gradients of shear stress as found using sudden-expansion parallel-plate flow chambers. An in vitro tissue model of human endothelium has been developed that supports cell culture over long time periods (hours to days) in a controlled fluid dynamics environment (steady or pulsatile flow). This system provides a large surface area of cells to be exposed to uniform shear stress as indicated by computational fluid dynamics modeling and micro-PIV experiments. These models may be used to generate large samples for microarray studies for gene expression, DNA-protein for chromatin immunoprecipitation studies for transcriptional regulation, and western blotting for protein levels. Also at Calgary, Leo Behie, Michael Kallos, and Arindon Sen are attacking the bottleneck problem preventing the development of stem cell-based therapies (and their subsequent widespread clinical implementation), namely, that these cells are present in very sparse quantities within tissues, and thus cannot simply be harvested for use in sufficient amounts. These researchers are finding ways to alleviate this bottleneck<sup>46</sup> by developing standard methods to scale-up and reproducibly generate clinical quantities of stem cells, thereby paving the way for clinicians to develop therapies to treat patients suffering from devastating diseases which have traditionally been deemed to be incurable. They currently have a number of medically relevant projects, which involve neural, mesenchymal, pancreatic, embryonic, and cancer initiating stem cells.<sup>47,48</sup> Specific projects include the development of serum-free media, the development of cell handling protocols, and the development of methods to scale-up cell production in suspension culture bioreactors.

Brian Amsden at Queen's University works with Millennium Biologix Inc., in developing a scaffold for articular cartilage regeneration, and in the delivery of a bone growth stimulating peptide. In related work, Stephen Waldman also

works with articular cartilage and ligaments, which are unable to repair themselves following damage. Improving tissue growth rates through bioreactor development and mechanical stimulation of the cells to modify physiological function are important aspects of the research. For example, stimulation has improved wear-resistance and resulted in a nearly frictionless surface characteristic of the articular cartilage. Kim Woodhouse at Queen's is developing biomimetic and synthetic polymeric materials for soft tissue engineering and regenerative medicine. A patented biodegradable polyurethane can be modified to specific cardiac tissue engineering applications. Formed through electrospinning technique, this fibrous polymer influences the ability of the cells to beat synchronously, simulating the response of cardiomyocytes in the heart. The cells have been derived from human embryonic stem cells. In addition, a patented family of elastin polypeptide-based materials are produced by recombinant techniques. Elastin is a structural protein found in soft tissues, and are being evaluated for vascular graft applications. A company is presently being formed to develop this technology. Also at Queen's, Lauren Flynn is a tissue engineer who is undertaking research on the use of naturally derived biomaterials for soft-tissue regeneration. She is also interested in the isolation and culture of adult stem cells derived from adipose tissue.

Kibret Mequanint's research at the University of Western Ontario is focused on vascular tissue engineering in an attempt to understand vascular smooth muscle cells (VSMC) interactions with polyurethane biomaterials. Recent studies have demonstrated that the phenotype of VSMC in both 2D and 3D polyurethane surfaces can be modulated using specific extracellular matrix components. The migration and proliferation of VSMC on porous 3D scaffolds has provided key information to progress in the design of engineered blood vessels.<sup>49</sup> In parallel, his group is also investigating different polyurethane biomaterials for use as nonthrombotic medical devices or device coatings.<sup>50,51</sup>

Roshni Dutton at Ryerson University centers her research efforts on the application of macroscale metabolic engineering techniques for the optimization of cell culture systems.<sup>52</sup> Dutton's work involves evaluation of the impact of variation in the metabolome on the proteome of cultured mammalian cells, as brought about through manipulation of the culture environment (medium, temperature, pH, etc). MFA and metabolic profiling (MP) are used to evaluate the metabolome of cultured mammalian cells through measurements of extracellular medium components and metabolites (amino acids, proteins, lactate, ammonia, etc). She has a particular focus on the development of mammalian cell culture systems producing macromolecules (proteins) having biopharmaceutical value. Monitoring, optimization, and control of mammalian cell culture systems is brought about via nutritional profiling using multivariate statistical analysis and the subsequent application of dynamic programming. Dutton also has an interest in extending these macroscale metabolic engineering techniques to include applications in the development of in vitro cell-culture based toxicology assays and in the development and optimization of animal-derived component free cell culture media, with particular interest in plant-derived components.

In Montreal at the Biotechnology Research Institute (BRI) of the National Research Council of Canada research on insect cell technology combines basic studies on the metabolism and physiology of Sf-9 insect cells during infection by

Baculovirus<sup>53</sup> with fundamental bioreactor engineering concepts together with advanced on-line monitoring and process control, the group at BRI has been able to obtain a substantial increase in recombinant virus titers (up to 10- to 100-fold increase over standard yields). This technology is not limited to recombinant protein production but is being seen in areas such as biopesticides, vaccines, and viral vectors for gene therapy. Mammalian cell technology is also being studied via the development of the HEK-293 cell line (human) and successful scale-up of HEK-293 cell-based processes in suspension and serum-free culture.<sup>54</sup> The central metabolism of HEK-293 cells has been characterized using MFA. Identification of limiting pathways led to genetic modifications of the cell line(s) to improve metabolism during growth and, more importantly, during product production. This work has led to the establishment of HEK-293 cells as an important second generation technological platform for the production of recombinant proteins and viral vectors using proprietary cell lines and processes. The group has also made several significant contributions to the field of on-line monitoring of mammalian cell physiology in real time during cultivation in bioreactors. In the area of viral vector and vaccine technology, researchers at BRI have been studying adenoviral vector production, research that included maximization strategies, advanced process control strategies, novel quantification and purification methods, and on-line monitoring tools.<sup>55</sup> The group succeeded in the development of robust cGMP-compliant bioprocesses for viral vector production to facilitate Canadian research on gene therapy. The group now operates the only nonindustrial gene vector production facility in Canada for research purposes, using dedicated facilities and proprietary technologies. In the last few years, the expertise has been applied to other viruses with gene therapy and vaccination potential (retrovirus, lentiviruses, reoviruses, and the adenovirus-associated virus).

At the University of Waterloo, a new area of research includes biomedical engineering and nanobiotechnology. Fundamentals in physical chemistry, surface thermodynamics, solid state physics, biochemistry, and molecular cell biology are expected to be applied to drug and gene delivery, peptide-DNA/RNA binding, protein-lipid interactions, lipid bilayer and cell membrane actions, and therapeutic lung surfactants. In addition adeno-associated viruses are being investigated as potential vectors for gene therapy of Alzheimer's disease, Parkinson's disease, prostate, and breast cancers.

### Separations

Raja Ghosh and Carlos Filipe at McMaster University are developing bioseparation strategies for purification of biopharmaceuticals such as monoclonal antibodies, PEGylated proteins, and hyperimmune equine and human polyclonal antibodies based on ultrafiltration and membrane chromatography, separately and in combination with conventional techniques such as precipitation.<sup>56</sup> Other applications include high-resolution separation and analysis of monoclonal antibody aggregates and antibody purification from transgenic tobacco plants. As well as the separation methods themselves, this work involves studies of membrane biofouling, process optimization, development of "smart" environment responsive membranes and membrane-based immunoassay techniques.<sup>57</sup> Some recent work focuses on development of membrane bioreactors for production of value-added bio-

pharmaceuticals. An example of the application of molecular biology in recombinant protein purification is based on a novel approach referred to as "inverse transition cycling."<sup>58</sup> In this approach, a fusion of the target protein with an elastin-like polypeptide (ELP) is expressed, e.g. in *E. coli*. Based on the solution phase behavior of the ELP, the fusion protein can be aggregated by an increase in temperature or salt concentration. The aggregates are then separated from the cell lysate using a membrane with a suitable cutoff. The aggregates are solubilized by reversing the phase transition (reducing the salt concentration or temperature), and the target protein is recovered in a subsequent step.

At the University of Saskatchewan, Catherine Niu has been developing biosorption processes using low-energy and cost-effective biomaterials to sequester chemicals of interest. She utilizes agricultural waste materials readily available in Saskatchewan to selectively remove water from the ethanol-water so as to concentrate ethanol. This technology can substantially reduce operating cost for the fuel alcohol production. Additionally, she also uses biomaterials to adsorb metals from aqueous solution. This technology could be applied in mining and hydrometallurgy for metal concentration in the process solutions and in environmental engineering for toxic metal treatment.

At the University of Waterloo research is being conducted on the development of efficient membrane filtration processes by combining modeling with experimental validation. In particular the examination of the fouling occurring during membrane microfiltration operation has confirmed the importance of the transmembrane pressure profile along the membrane module. Adjustment of the pH of soy protein extracts by electroacidification followed by concentration using tangential flow membrane ultrafiltration has also been shown to improve mineral and phytic acid removal resulting in a better quality product. The potential of spectrofluorometry for data acquisition and development of soft sensors for the estimation of process variables appears to be very promising.

B. Q. Liao at Lakehead University has been focusing on environmental biotechnology and bioseparations. More specifically, membrane technology is incorporated into biological processes to simplify unit operations, enhance productivity, and improve product quality.<sup>59</sup> At present, various types of membrane bioreactors, including aerobic membrane separation bioreactor, membrane aerated biofilm reactor, and anaerobic membrane separation bioreactor, are being studied at Lakehead, in terms of optimal design of membrane modules and process conditions, membrane fouling control, and new applications.<sup>60</sup>

Robert Legros at Ecole Polytechnique in Montreal has been developing integrated downstream processes using expanded bed adsorption (EBA). In several cases, biomolecules of interest produced from cell cultures in bioreactors (e.g. plant cell secondary metabolites) have inhibitor effects on the culture or have short half-life. It becomes important then to remove these bioproducts as rapidly as possible from the culture environment. The objective of the research is to develop efficient downstream processes for in situ capture systems to be integrated with the bioreactor. The research also involves the characterization of EBA columns (mass-transfer and hydrodynamics modeling, determination of scale-up criteria) and the development of novel adsorbant resins for integrated capture systems.

Yaser Dahman at Ryerson University is undertaking research in the bioseparation field, with focus on the primary

recovery of high-value proteins and industrial enzymes from fermentation broths. This includes the design and synthesis of novel functionalized micro- and nano-spherical polymeric resins, in addition to functionalized cellulosic nanofibers membranes.<sup>61</sup>

Charles Haynes, in collaboration with other UBC scientists and Merck Inc., has developed a number of new chromatographic modes and resins to efficiently separate biomolecules on the basis of size, charge, or hydrophobicity, which have now become standard in the bioprocessing industry. He has developed a number of other protein and nucleic-acid capture and purification technologies for nanoscale to manufacturing-scale applications. These include controlled-shear affinity filtration technology for recombinant protein purification, the CBinD™ affinity chromatography cassette system (with Doug Kilburn), and TEMplex™ technology, which is now a widely used system that removes a former bottleneck in high-throughput DNA sequencing.

### Conclusions

The present survey has provided a “snapshot” that describes research activities in bioengineering in Canada in mid-2007. The areas under investigation and the level of research intensity in each area no doubt reflect the interests of individual researchers, as well as influences arising from the strategic hiring practices and directions of individual departments, and the funding priorities of government and private sector partners. Based on the crude metric of number of researchers involved in a particular subtopic, rather than, for example, research support or publication output, it would appear as though environmental biotechnology, biomaterials, and tissue/cell culture engineering are the most active areas at the moment.

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