# Center For Biomedical Engineering

## Executive Summary

The mission of the Center for Biomedical Engineering (CBE) is to combine engineering with molecular and cellular biology to develop new approaches to biomedical technology with applications to medicine and biology. CBE continues to play a lead in MIT's activities in tissue engineering and therapeutic approaches for applications in the repair of musculoskeletal and cardiovascular tissues. Recent initiatives have focused on the delivery of drugs to stop the progression of osteoarthritis associated with traumatic joint injuries. New collaborations with the Harvard School of Dental Medicine, Boston University Medical School, and Harvard Medical School have focused on the generation of two new Boston-area based centers for translational research in osteoarthritis. In addition, fundamental discoveries in mechanobiology and drug delivery by CBE faculty and students have enabled critical advances in tissue remodeling and regeneration.

To maintain intellectual leadership in the still-rapidly growing biotechnology industry-academic paradigm, both in Boston and nationwide, innovative approaches are needed to stimulate fundamental research and to facilitate timely translation of new discoveries into health care sectors. With these goals in mind, CBE continues to identify opportunities and connections with industry that are aligned with its core research areas. The center continues to maintain a well-used base of core research facilities that are provided at minimal user cost to the MIT community. Taken together, our aim is to pursue multidisciplinary biomedical research and create an outstanding training environment for a new generation of students/leaders in biomedical and biological engineering.

## Major Research Areas

CBE's core faculty members represent a variety of academic units, primarily within the School of Engineering, but with substantial participation from School of Science faculty and collaborating faculty from Harvard Medical School; Colorado State University Medical School; Harvard School of Dental Medicine; Boston University School of Medicine and Department of Rheumatology; Lund University Department of Clinical Sciences, Sweden; and the Laboratory for Molecular Biomechanics and Clinic for Orthopaedic and Trauma Surgery, Tübingen, Germany. These faculty participate in multi-investigator programs focusing on CBE's primary research areas:

- cell and tissue engineering
- biomaterials and hydrogel scaffolds for regenerative medicine and drug delivery
- membrane protein structural biology, design, and fabrication
- mechanobiology and the underlying cellular mechanisms by which traumatic injuries cause degenerative diseases in connective tissues

Together, these research areas have direct applications to musculoskeletal and cardiovascular pathophysiology, tissue regeneration and repair, drug discovery, environmental and biohazard sensors based on membrane proteins, and

nanobiotechnology. CBE maintains a broad funding base with support from the National Institutes of Health, the National Science Foundation, and the Department of Defense, as well as industry (primarily pharmaceutical). CBE faculty members participate in many interdepartmental programs as well as collaborative interactions with other universities and industry research laboratories.

## Major Research Initiatives

A major initiative has focused on the treatment of patients who suffer from traumatic joint injury, a condition with very high risk for developing osteoarthritis. About 27 million Americans suffer from arthritis, with more than three million of those cases resulting from a joint injury, often in the knee, that provokes slow and steady cartilage deterioration. Severe joint injuries are more common in younger people, who are more likely to participate in sports and military service. In the knee, injuries often involve tearing the meniscus and ligaments such as the anterior cruciate ligament (an injury also common in older adults). In most cases, patients are treated first with non-steroidal anti-inflammatory drugs to reduce pain and swelling. Weeks or months later, they might have surgery to stabilize the joint, but in about 50 percent of cases, the patient's cartilage steadily breaks down after the injury, leading to osteoarthritis. The risk of progression to osteoarthritis is known to exist with or without reconstructive surgery.

The new CBE study, in collaboration with the Beth Israel Deaconess Medical Center and Harvard Medical School, suggests that, if given soon after the injury, a glucocorticoid drug currently used to treat inflammatory diseases could also prevent osteoarthritis from ever developing in these patients. The drug, dexamethasone, has been prescribed to treat chronic rheumatoid arthritis in the elderly for decades.

CBE researchers tested the effects of dexamethasone on human and bovine cartilage tissue in vitro. The cartilage was first injured mechanically in a manner simulating the effects of a human joint injury, and then cultured with inflammatory proteins called cytokines, which are typically released by cells in joint tissues after a human joint injury. These cytokines cause accelerated cartilage breakdown. In damaged tissue treated immediately with dexamethasone, cartilage breakdown was halted. The drug also worked when given a day or two after the injury, which is important because people who suffer joint injuries might not get to see a doctor right away.

Animal studies are being planned to test this approach in vivo, and to determine how many joint treatments may be necessary to maintain the protective effect. If those studies yield positive results, the findings could be rapidly translated to human treatments since the drug is already approved for human use. Studies on appropriate drug delivery localized to the joint, as well as the mechanisms associated with the protective effect, are now ongoing. This initiative was featured in an MIT press release earlier this year.

In another initiative, CBE investigators have extended research on self-assembling peptide scaffold design and functionalization for advanced applications in cell culture, regenerative medicine, and drug delivery. In one series of studies, two peptide scaffolds were designed and developed for periodontal ligament fibroblasts. The scaffolds consist of one of the members of the self-assembling peptide family, RADA16, directly

coupled to short biologically active motifs. The motifs are 2-unit RGD binding sequence PRG (PRGDSGYRGDS) and the laminin cell adhesion motif PDS (PDSGR). RGD and laminin have been previously shown to promote specific biological activities including periodontal ligament fibroblast adhesion, proliferation, and protein production. Compared to the pure RADA16, the new designer peptide scaffolds promote a significant increase in proliferation and migration of human periodontal ligament fibroblasts into the scaffold. Moreover, these peptide scaffolds stimulated periodontal ligament fibroblasts to produce extracellular matrix proteins without using extra additional growth factors. Immunofluorescent images demonstrated that the peptide scaffolds were almost completely covered with type I and type III collagen, which are the main protein components of periodontal ligaments. These results suggest that such designer self-assembling peptide nanofiber scaffolds may be useful for promoting wound healing, and especially periodontal ligament tissue regeneration.

In a related endeavor, a new chiral self-assembling peptide, d-EAK16, was designed and analyzed for tissue engineering studies. A rabbit liver wound-healing model was investigated to examine rapid hemostasis. It was found that only about 20 seconds was needed to achieve hemostasis using 1% d-EAK16. Thus, a model was successfully developed to understand the chiral assembly properties for rapid hemostasis and to aid in further design of self-assembling peptide scaffolds for clinical applications.

CBE researchers have joined an initiative to form a Boston-area Center of Research Translation (CORT). The theme of this center is the study of the effect of biomechanics on the clinical course and treatment of osteoarthritis. The center comprises experts from Boston University Medical School, Children's Hospital and Massachusetts General Hospital (both of Harvard Medical School), and CBE investigators. CORT project areas focus on direct patient-clinical interest,, including:

- a clinical trial testing a lateral wedge shoe insert for the treatment of medial knee osteoarthritis, which will involve gait studies and the potential for prescription of biomechanical therapy to optimize efficacy
- study of changes in bone shape that occur with the development of osteoarthritis and the relationship between bone shape and altered biomechanical stresses across the joint
- a study of human hip dysplasias which result from biomechanical stress, along with surgery to correct this dysplasia

In particular, the hip dysplasia problem, referred to as femoroacetabular impingement, is a highly prevalent deformity of the hip joint that may lead to hip dysfunction and osteoarthritis. Thus, the purpose of this latter study is to help confirm the importance of pathomechanics as a cause of abnormal cartilage mechanics in vivo, to identify biochemical changes that may be occurring in these joints that may contribute to progression of chondral damage, and to identify in vivo hip and spine movement patterns that may contribute to making some hips symptomatic. Investigators from CORT-affiliated institutions meet each month to plan initial studies.

Exciting advances have been made in another ongoing research area: that involving membrane proteins and a class of G-protein coupled receptors (GPCRs). GPCRs are

involved in a wide range of vital regulations of human physiological actions. They are also of pharmaceutical importance and have become therapeutic targets for a number of disorders and diseases. Purified GPCR-based approaches, including structural studies and novel biophysical and biochemical functional analyses, are increasingly being used in GPCR-directed drug discovery.

Before these approaches become routine, however, several hurdles need to be overcome, including over-expression, solubilization, and purification of large quantities of functional and stable receptors on a regular basis. The CBE team succeeded in milligram production of a human formyl peptide receptor 3 (FPR3), which comprises a functionally distinct GPCR subfamily that is involved in leukocyte chemotaxis and activation. The bioengineered FPR3 was over-expressed in stable tetracycline-inducible mammalian cell lines (HEK293S). After a systematic detergent screening, fos-choline-14 was selected for subsequent solubilization and purification processes. A two-step purification method, immunoaffinity using the anti-rho-tag monoclonal antibody 1D4 and gel filtration, was used to purify the receptors to near homogeneity. Immunofluorescence analysis showed that expressed FPR3 was predominantly displayed on cellular membrane. Secondary structural analysis using circular dichroism showed that the purified FPR3 receptor was correctly folded in a manner similar to other known GPCR secondary structures. Thus, the developed method can readily produce milligram quantities of human FPR3, which would facilitate the development of human FPR as therapeutic drug targets.

In a related study, CBE investigators studied the selection of the right surfactant for producing GPCRs. Peptide surfactants have been used in commercial *Escherichia coli* cell-free systems to rapidly produce milligram quantities of soluble GPCRs. These include the human formyl peptide receptor, human trace amine-associated receptor, and two olfactory receptors. The GPCRs expressed in the presence of the peptide surfactants were soluble and had $\alpha$-helical secondary structures, suggesting they were properly folded. Microscale thermophoresis measurements showed that one olfactory receptor expressed using peptide surfactants bound its known ligand heptanal (molecular weight 114.18). These short and simple peptide surfactants may be able to facilitate the rapid production of GPCRs, or even other membrane proteins, for structure and function studies.

CBE investigators have continued research on the development of growth factors containing specific binding regions that enable retention and sustained delivery of these bioactive proteins to one or several specific tissues within the body. In a collaboration involving Brigham and Women's Hospital, IPSEN Pharmaceuticals, the Massachusetts Life Sciences Center, and CBE, investigators have thus far focused on insulin-like growth factor-1 (IGF-1), which stimulates tissue growth and cell biosynthesis of tissue extracellular matrix. The use of IGF-1 for musculoskeletal connective tissue repair is a particularly important application, though not a practical therapy when the growth factor is delivered systemically, due to rapid release of the drug from the body and potential side effects in non-targeted tissues.

The CBE team modified IGF-1 by adding a heparin-binding domain and showed that this fusion protein, HB-IGF-1, stimulates sustained extracellular matrix synthesis in tissues with a matrix containing a high negative charge density, such as cartilage and

the meniscus. New animal studies have now shown that intra-articular injection of HB-IGF-1 into rat knee joints leads to sustained retention for up to eight days, and bioactivity has been demonstrated for a minimum of four days. While electrostatic interactions help to augment partitioning of this positively charged drug into tissues with a high negative charge, such proteins would rapidly diffuse out of the tissue unless they could specifically and reversibly bind to sites near the tissue cells. Such binding enables local depot delivery of the drug. This discovery suggests that modification of growth factors with heparin-binding domains may be a new strategy for sustained and specific local delivery to a range of musculoskeletal and cardiovascular tissues.

## Center for Biomedical Engineering Core Facilities

One of the critically important missions of CBE is to maintain and expand a set of central core research facilities. These core facilities are made available to faculty, staff, and students at no or minimal user cost, and are particularly relevant for CBE's major research areas. Facilities include:

- an Attana 200 dual channel, label-free, temperature controlled, continuous-flow system for analysis of molecular interactions (e.g., kinetics, affinity and off-rate screening)
- a Rigaku X-ray diffraction facility for protein and molecular crystallography and analysis
- the widely used Applied Biosystems 7900HT fast real-time 384-well plate quantitative polymerase chain reaction (PCR) instrument and associated peripherals
- a NanoDrop ND-1000 Spectrophotometric Analyzer to measure quality of RNA which is useful in assessing samples prior to amplification using the AB 7900HT qPCR facility
- an Alpha Innotech Gel Imaging Facility has been added for quantitative analysis of electrophoresis gels
- a multiphoton microscopy facility
- a Cressington Quick-Freeze Deep Etch facility to prepare specimens for follow-on electron microscopy
- a BiaCore 2000 surface plasmon resonance instrument to quantify binding reaction constants between molecules and between molecules and surfaces
- a large cell, tissue, and organ culture facility including four 6-foot biosafety cabinets and eight incubators

These are available to faculty and students who would otherwise not be able to explore new ventures in biomedical engineering involving living cells because of a lack of specialized facilities in their own laboratories (including an array of associated instruments and peripherals needed for maintaining and experimenting with living cells and tissues).

## Undergraduate Research Opportunities Program Activities

CBE continues to connect outstanding undergraduate students in several departments to laboratories associated with CBE and at the Beth Israel Deaconess Medical Center, working in cancer-related research. This is a long-standing partnership in which CBE provides logistical and administrative support to ensure continued success of this interaction.

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