Laboratory Courses Focused on Tissue Engineering Applications

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Abstract

Two new laboratory courses focused on tissue engineering have been developed and implemented in the undergraduate Bioengineering Department at Rice University. The content of these courses is quite unique, yet fully supports the department's emphasis on biomedical engineering at the molecular, cellular, and tissue levels. This sequence of required laboratory courses is designed to teach students mammalian tissue culture techniques, to develop their ability to design and conduct experiments, and to meet other department-specific ABET Program Outcomes. In BIOE 342, students learn sterile technique, develop their ability to visually assess cell morphology and confluency, and learn how to maintain cells in culture. Using fibroblast cells, students conduct viability, attachment, and proliferation assays. Differences in surface properties and serum concentrations are explored in the attachment and proliferation assays, respectively. Quantitative techniques to measure cell number and activity are stressed. One module within BIOE 441 focuses on the application of tissue culture techniques to a tissue engineering challenge. Students assess the physical and chemical properties of the polymer, poly(L-lactic acid) (PLLA), and complete a four-week degradation study of PLLA films. Students design and conduct experiments to quantify the viability, attachment, and proliferation of fibroblast cells on PLLA films. Students are not given protocols for these experiments; they must develop new protocols or adapt protocols from BIOE 342 and make adjustments for differences such as cell seeding levels, attachment kinetics, and proliferation rates. Most students repeat experiments with a redesigned protocol. Because of the open-ended nature of the assignment, students develop unique approaches and protocols. Contents of the laboratory courses, assessment of the courses, equipment start-up and operating costs, and dissemination of course materials are discussed.

Bioengineering Curriculum at Rice University

The Bioengineering undergraduate program at Rice University is designed to prepare students for careers in the rapidly developing areas of biomedical engineering and bioprocessing. The undergraduate educational program in Bioengineering has the goal of producing a new type of biomedical engineer, fully conversant with modern biochemistry and cell and molecular biology.[1-2] This type of biomedical engineer translates bench-scale scientific advances in biological sciences into cost-effective new products and processes. New and innovative curricula in lecture and laboratory courses are being developed to educate bioengineers who not only create new tissues and cell-based therapies but also deliver them at a cost affordable to our health care system.

The educational program objectives of the B.S. degree in Bioengineering at Rice University are to:

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- Provide students with a fundamental understanding of mathematics and the natural, life, and medical sciences;
- Teach students bioengineering principles and their applications in the life and medical sciences;
- Develop their critical problem solving skills in bioengineering;
- Develop their ability to communicate effectively and participate in interdisciplinary teams;
- Expose students to a broad education that prepares them for diverse careers.

Students obtaining a B.S. in Bioengineering are required to take general technical courses, Bioengineering core courses, and Bioengineering track elective courses. General technical courses include Calculus (4 semesters), General Chemistry (2 semesters), Physics (2 semesters), Organic Chemistry (2 semesters), Introductory Biology (2 semesters), Biochemistry (1 semester), Cell Biology (1 semester), Computational Mathematics (2 semesters), and Engineering Mechanics (1 semester). The Bioengineering core courses (1 semester each) include Conservation Principles in Biology and Medicine, Biosystems Transport and Reaction Processes, Systems Physiology, Biomechanics and Biomaterials, Thermodynamics, Tissue Culture Laboratory, Bioengineering Design, and Advanced Bioengineering Laboratory. Students select one of three emphasis areas or tracks: (a) Cellular and Molecular Engineering, (b) Bioinstrumentation, Imaging, and Optics, or (c) Biomaterials and Biomechanics. Students take a minimum of five elective courses that expose them to important problems in their chosen track.

The curriculum in Bioengineering requires a number of laboratory courses beginning at the freshman year with General Chemistry and Physics laboratory courses. At the sophomore year, students are required to take Organic Chemistry Lab. In addition, most students take BIOS 211, Introductory Lab Module in Biological Sciences, which focuses on the scientific method, principles of experimental design, selected research strategies, record keeping, and technical communication. In the junior year, students take BIOS 311, Lab Module in Protein Purification, or BIOS 312, Lab Module in Molecular Biology. BIOS 311 provides experience in the manipulations, instrumentation, and considerations for purifying proteins. BIOS 312 provides an introduction to microbiological and molecular biology techniques. Within the Bioengineering Department, students take BIOE 342, Lab Module in Tissue Culture, during their junior year and BIOE 441, Advanced Bioengineering Laboratory, during their senior year.

Goals of Bioengineering Laboratory Courses

The laboratory environment is well suited for hands-on training in bioengineering. Laboratory courses are ideal for students to refine their abilities to design and conduct experiments, as well as to analyze and interpret data. Students also learn modern biological methods and engineering applications. Finally, assignments can easily be tailored to enhance communication skills. Below is a table that lists the eleven Program Outcomes for the Rice Bioengineering Department. BIOE 342 and BIOE 441 contribute substantially to fulfilling the Program Outcomes. Only outcomes for the Tissue Engineering (TE) Module of BIOE 441 are listed.

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ABET Program Outcomes (Abbreviated) Rice University Bioengineering Department	BIOE 342	BIOE 441 TE
1. Knowledge of basic science, engineering fundamentals and life sciences	X	X
2. Ability to identify, formulate and solve breadth of bioengineering problems		
3. Ability to design and conduct experiments, analyze and interpret data	X	X
4. Ability to use techniques, skills and modern engineering tools	X	X
5. Ability to design a system or component by synthesizing knowledge		X
6. Ability to solve advanced bioengineering problems in one emphasis area		X
7. Ability to communicate to technical and non-technical audiences	X	X
8. Ability to work effectively in multi-disciplinary teams	X	X
9. Understanding of the professional and ethical responsibilities		
10. Education that includes opportunities for "out-of-classroom" learning		
11. Education that prepares for post-graduate education and life-long learning		X

Many lower-level laboratory courses at Rice University and other universities are designed to illustrate a scientific concept or engineering principle or to teach students basic laboratory skills. In addition, laboratory courses with freshman and sophomore students (e.g. introductory Physics, Chemistry, and Biology laboratory courses) often have high enrollments. Instructors often develop course-specific protocols; many protocols are published on the Web[3-5] or in journals (e.g. *Journal of Chemical Education*, *Chemical Engineering Education*, and *Biochemical Education*). In BIOE 342 and the "PLLA and PLGA Characterization" segment of the Tissue Engineering Module in BIOE 441, detailed protocols are appropriate and are utilized.

Open-ended projects are very common in senior capstone design courses across all engineering disciplines. In biomedical engineering and bioengineering departments is it common to have a one- or two-semester design course that involves a paper or computer-aided design as well as building a device or product in the laboratory. Many students then experience the trial-and-error nature of experimental work in their efforts to build or design a device or product. Undergraduate research is also a venue for students to learn to design laboratory experiments.

Less common is the integration of open-ended projects and student-driven experimental design in a laboratory course. However, several departments and programs are making contributions in this area including the Colorado School of Mines Multidisciplinary Engineering Laboratory Courses,[6] the VaNTH Engineering Research Center program,[7] and others.[8] To meet the ABET Program Outcomes in BIOE 441, the "Interaction of HDF Cells with PLLA Films" segment of the Tissue Engineering Module in BIOE 441 was developed to allow students the opportunity to design experiments to meet specific technical objectives.

Lab Module in Tissue Culture (BIOE 342)

Lab Module in Tissue Culture (BIOE 342) has been taught in Spring 2001 and Spring 2002. Students receive 1 hr of credit for BIOE 342. Depending on class enrollment, three or four sections of BIOE 342 are taught each spring semester. Each section lasts 5 weeks; sections start at the beginning of the semester and after spring break. The class meets three times a week; sections are offered Monday/Wednesday/Friday (M/W/F) and Tuesday/Thursday/Saturday

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(T/T/S). Class times on M/W and T/T are 1:00 - 6:00 p.m. On F and S, students need to be in the lab less than 1 hr (usually to feed and/or passage cells).

In BIOE 342, students learn and master sterile technique. They develop their ability to visually assess cell morphology and confluency. Students learn how to maintain cells in culture and are responsible for maintaining cells throughout the course. Students conduct a range of experiments, including viability, attachment, and proliferation assays. Students also transfect cells with a plasmid containing green fluorescent protein (GFP). Quantitative techniques to measure cell number and activity are stressed. Human Dermal Fibroblast (HDF) cells (gift from Dr. Jennifer West at Rice University) are used in most of the experiments. Fibroblasts are used since they are reasonably hearty. Other types of fibroblasts, such as 3T3 cells, could be used.

Detailed protocols developed by the author are bound and made available to the students. Other handouts developed by the author such as "Tips for Sterile Technique and Cell Manipulation" supplement the protocols. Reading assignments are made from the text, Culture of Animal Cells: A Manual of Basic Technique.[9] The author presents weekly 1 hr lectures.

The experiments in this course are divided into three segments: "Introductory Material," "HDF Cell Survival and Function," and "GFP Transfection." The scheduling of the experiments is shown in Appendix 1. The scope and/or goals of each experiment are given below.

Introductory Material

The protocols in this segment are designed to familiarize students with the equipment and tools used in tissue culture work. Experiments in this segment are short (1-3 hrs), and all six can be completed in 2-3 afternoon sessions.

- Micropipette Exercise - Students become comfortable using micropipettes. Using a scale, the density of water is calculated using several different aliquot volumes.
- Contamination Exercise - Using agar plates, students test air exposure in different locations of the lab (laminar flow hood, bench), water from different sources, yogurt, etc. for presence of bacterial and fungal contaminants. An exercise with *S. marceans* demonstrates that bacteria can be present, even when it initially can not be seen.
- pH and Color of Media - Using vials of media prepared at different pH values, students develop visual recognition of media conditions that are optimal for cell growth.
- Visualization of Cells - Students visually assess confluency, degree of attachment, and morphology of several types of cultured cells. Students become familiar with microscopes and learn importance of visual assessment.
- Sterile Technique - Students practice and learn the sterile procedures necessary to keep cells alive and growing under cultured conditions. Students practice liquid transfer, feeding cells, and passaging (splitting) cells on tables under non-sterile conditions in the lab. Students coach one another and identify technique mistakes that could lead to contamination.
- Determination of Cell Concentration - Students determine the concentration of a cell suspension using both a hemocytometer and Coulter Counter.

HDF Cell Survival and Function

Viability is explored in two assays in this segment. Cell attachment and proliferation are each explored in a qualitative and quantitative assay. This redundancy allows students to compare

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results as well as to see the value of quantitation. Several experiments in this segment take up to 6 hrs in one session; others take 2-3 hrs spread over 3-4 sessions. The experiments in this segment are spread over 8 afternoon sessions.

- Live/Dead Fluorescence Assay - Students use the Live/Dead reagent (Molecular Probes) to visualize HDF cells. Students become familiar with the fluorescence microscopes.
- MTT Viability Test - Students use the MTT assay (Promega) to develop a linear relationship between absorbance of MTT dye measured using a spectrophotometer and concentration of HDF cells.
- Fibronectin Attachment Assay - Students qualitatively assess the attachment of HDF cells to untreated and fibronectin-coated surfaces after a 2 hr period. Students use paint brushes to coat wells and paint designs with fibronectin.
- Quantitative Cell Attachment Assay - Students count the number of attached HDF cells four times over a 4 hr period to TC-treated, untreated, and fibronectin-coated surfaces. Statistical comparisons are made among different surface treatments to compare attachment kinetics.
- Anti-PCNA Staining - Students culture HDF cells in media with different serum concentrations (1%, 5%, 10%). The Proliferating Cell Nuclear Antigen (PCNA) is used as a marker for cell proliferation. Cells that contain PCNA are stained using an antibody to PCNA with Horseradish Peroxidase (HRP).
- Cell Proliferation Assay - Students monitor the growth of HDF cells in media with different serum concentrations (1%, 5%, 10%). Cell number is measured initially and at 2, 5, and 7 days after seeding; this allows students to see the lag, exponential, and plateau phases. Cell doubling time is calculated for each treatment.

GFP Transfection

The steps in this experiment take 2-4 hrs and are spread over 4 sessions.

- GFP Transfection Assay - Students transfect Chinese Hamster Ovary (CHO) cells with a plasmid containing Green Fluorescent Protein (GFP) using Lipofectamine (Gibco). Cells containing the plasmid are selected using antibiotic resistance. Cells containing GFP are visualized using a fluorescence microscope.

The assignments and grading for the course are geared to reinforce laboratory work and analysis of collected data. On a weekly basis, students turn in carbon-copies of their lab notebooks (15% of grade). Proper form and notation are graded. At the end of most protocols are questions that the students must answer (30% of grade). Most questions focus on observation, analysis, statistical comparison between treatments, or comparison between experiments. Students are required to put together a draft and a final poster that cover the objectives, succinct summary of experimental methods, experimental results, and implications of the work from the "HDF Cell Survival and Function" segment of the course (35% of grade). Finally, students are graded on their tissue culture skills (15% of grade) and safety in the lab (5% of grade).

The instructor works collaboratively with staff from the Cain Project in Engineering and Professional Communication on the poster project. Staff from the Cain Project provide guidance to the students for the design of a technical poster and participate in critiques of the draft posters. Students also critique one another's posters. Noticeable improvements in style, content, and organization are seen when comparing the draft and final posters.

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Advanced Bioengineering Laboratory (BIOE 441)

Advanced Bioengineering Laboratory (BIOE 441) was taught for the first time in Fall 2001. Students receive 4 hrs of credit for BIOE 441. Two sections (M/W and T/T) of BIOE 441 are offered each fall semester. Each section lasts 15 weeks. Class times on M/W and T/T are 1:00 - 6:00 p.m. On F and S, students may need to be in the lab to attend to their designed cell experiments. Students work in groups of two.

BIOE 441 is comprised of five different modules through which the students rotate. The modules are listed with a short description:

- Biomechanical Testing of Chicken - Using the Instron, students conduct three-point flexure and compression of bones, preconditioning and tensile failure of skin, and stress-relaxation and tensile failure of tendon.
- Tissue Engineering - See details below.
- Laser Tweezers - Using the Laser Tweezers in Dr. Bahman Anvari's laboratory, students conduct experiments to elucidate the detachment force of fibronectin-integrin bonds.
- Ethics - Students participate as stakeholders charged with exploring the NIH Guidelines for research using pluripotent stem cells.
- Biopac - Using the Biopac software and various medical measurement tools, students conduct several physiological tests including ECG, EEG, pulse, and pulmonary function.

The experiments in the Tissue Engineering Module comprise several of the basic experiments that would be required to do a preliminary investigation of a tissue-engineered product.[10-12] Students synthesize poly(L-lactic acid) (PLLA), characterize PLLA and poly(DL-lactic-*co*-glycolic acid) (PLGA), and monitor PLLA and PLGA degradation. In addition, students assess the viability, attachment, and proliferation of HDF cells on PLLA films. While protocols for some of the experiments are provided, protocols for the experiments involving HDF cells are not made available. Students must design and adapt their own protocols based on skills learned in BIOE 342 and other laboratory courses. The Tissue Engineering Module lasts 12 sessions (6 weeks).

The experiments in the Tissue Engineering Module are divided into two segments: "PLLA and PLGA Characterization" and "Interaction of HDF Cells with PLLA Films." The scheduling of the different experiments within these segments is shown in Appendix 2. The scope of each experiment and/or student ideas are given below.

PLLA and PLGA Characterization

The protocols in this segment focus on the characterization of PLLA and PLGA polymers using a number of different tools. PLLA and PLGA are purchased from Polysciences; in addition, students synthesize PLLA. Several experiments in this segment take up to 4 hrs in one session; others take 1-2 hrs spread over many sessions. The experiments in this segment are concentrated in 6 sessions, although the degradation experiment lasts 12 sessions.

- PLLA Synthesis - Students synthesize PLLA from L-lactide monomer with stannous octoate as the catalyst. Since the synthesis is conducted with heating under vacuum, students set up and operate a vacuum apparatus. The PLLA is purified by precipitation in methanol.

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- Prepare PLLA and PLGA Films - Students cast thin polymer films of PLLA and PLGA on glass coverslips.
- Characterization Using GPC - Students run the GPC to determine the molecular weight of various PLLA and PLGA samples.
- Characterization Using DSC - Students run a Differential Scanning Calorimeter (DSC) trace to identify the melting and glass transition temperatures of PLLA and PLGA.
- Characterization Using FTIR - Students run Fourier Transform Infrared (FTIR) to identify functional groups on PLLA and PLGA.
- Degradation of PLLA and PLGA - PLLA and PLGA films are incubated in PBS at 37 ˚C with gentle shaking. Weight, appearance, and molecular weight of both polymers are monitored over 4 weeks.

Interaction of HDF Cells with PLLA Films

This segment of the Tissue Engineering Module simulates "real-world" research and industrial environments. Students are given the task of demonstrating the viability, attachment, and proliferation of HDF cells on PLLA films. Students must design their own experiments, including the number of repeat tests, type and number of controls, cell seeding concentrations, test surfaces, etc. While many of the experiments parallel those in BIOE 342, several differences are also critical. For example, the well plate size is different. In addition, students have to attach the glass coverslips coated with PLLA to the bottom of the well plates and manage with some complications that arise from that procedure. Students have to do substantial planning. For example, students have to manage their flasks of HDF cells so that the cells are confluent on the days that they need cells for particular experiments. They also have to decide how many PLLA films they need and prepare them to be ready at the appropriate times.

Because of the open-ended nature of the assignment, students develop unique approaches and protocols. Most students repeat one or two experiments with a redesigned protocol. Several experiments in this segment take up to 6 hrs in one session; others take 1-2 hrs spread over 3 sessions. A group that plans well can accomplish all necessary work in 3-4 sessions. Six sessions are available for these experiments.

- Film Attachment and Sterilization - Students cast PLLA films and attach them to 12-well plates using vacuum grease. Plates containing films are sterilized under UV light.
- HDF Viability - Students must test the viability of HDF cells on PLLA films at 1-2 days in culture. Students typically used the Live/Dead reagent. The students designed various controls and conducted the assay with various degrees of quantitation.
- HDF Attachment - Students must demonstrate the attachment of HDF cells on PLLA films as a function of time during 4-6 hrs of culture. Students must compare attachment of HDF cells on PLLA films with appropriate control surfaces. Students struggled with low attachment fraction on the PLLA films, and many groups had to repeat the experiment with a higher initial cell seeding density. Students had difficulty visualizing the cells attached to the uneven PLLA surfaces. Some groups coated the PLLA films with fibronectin to accelerate attachment rates. Controls selected by the groups included TC-treated, untreated, and glass surfaces.
- HDF Proliferation - Students must demonstrate the proliferation of HDF cells on PLLA films during 5-7 days of culture. Students must compare proliferation of HDF cells on PLLA films with appropriate control surfaces. Students had difficulty removing the cells from the PLLA

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films for quantitation in the Coulter Counter. One group worked around this by adapting the MTT assay. Students struggled with different seeding densities necessary to achieve exponential growth of the cells during the allotted 5-7 day window. While several groups did report reasonable data, several groups failed to troubleshoot the many complexities of this experiment.

Each student writes a research paper summarizing the methods and results of the Tissue Engineering Module. A draft and a final research paper are graded. The papers are evaluated on clarity of writing, design of experiments, presentation of data, and analysis and interpretation of data. Extensive comments are made on the draft paper and are returned to the student within one week.

Assessment of BIOE 342 and BIOE 441

Since both courses are very new, assessment tools are still being developed. Current assessment includes extensive written terminal surveys and interviews that focus on content, laboratory experience, and lectures. The instructor also gauged the effectiveness of the two laboratory modules in terms of meeting the established ABET Program Outcomes using a range of 1 (low) to 5 (high).

ABET Program Outcomes (Abbreviated) **Rice University Bioengineering Department**	**BIOE 342**	**BIOE 441 TE**
1. Knowledge of basic science, engineering fundamentals and life sciences	4	5
3. Ability to design and conduct experiments, analyze and interpret data	2	4
4. Ability to use techniques, skills and modern engineering tools	4	4
5. Ability to design a system or component by synthesizing knowledge		4
6. Ability to solve advanced bioengineering problems in one emphasis area		4
7. Ability to communicate to technical and non-technical audiences	3	4
8. Ability to work effectively in multi-disciplinary teams	5	5
11. Education that prepares for post-graduate education and life-long learning		5

The instructor could see profound changes in the students' abilities to perform sterile technique and correctly conduct tissue culture assays during the course. In careful review of their lab notebooks and posters in BIOE 342, the instructor was able to observe that many students had difficulty analyzing their data in a rational manner and presenting their data in an appropriate way (ABET Outcome 3). Often, graphs were poorly constructed and inappropriate conclusions were drawn from the data. To address these deficiencies, increased one-on-one feedback was given to each student regarding her/his lab notebook and poster. Significant improvements in style, organization, and technical clarity were made between the draft and final posters (ABET Outcome 7). The students worked well together and were very capable using the equipment in the laboratory (ABET Outcomes 4 and 8).

Overall, students responded very favorably to BIOE 342. Most liked the experiments that they could "see" (e.g. experiments using fluorescence, HDF Attachment). Based on student evaluations, the focus and grading criteria of several assignments has shifted and the evaluation of tissue culture skills is more thorough.

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In the BIOE 441 Tissue Engineering Module, students had the unique opportunity to design and execute experiments that they designed (ABET Outcomes 3 and 5). While some students did well in the design and planning phase, other students performed poorly. In future years, it will be required to turn in an experimental plan prior to beginning any actual experiments. An improvement was definitely noted regarding the students' abilities to analyze and present their data in a clear and cohesive manner as compared to their skills in BIOE 342 (ABET Outcome 3). Again, extensive one-on-one feedback on the draft research paper was critical to significant student improvement (ABET Outcome 7). Changes in the due dates will improve effectiveness in the future. Students demonstrated a very high level of technical competence (ABET Outcomes 1 and 6).

Almost all of the students commented that they liked the freedom to design experiments and work through the protocols in a trial-and-error fashion. Many recognized this as an important skill they will need when they graduate (ABET Outcome 11). Next year, the hints for designing the experiments may be more limited. Many students also commented that they liked making the PLLA polymer. Complaints about the long hours (i.e., HDF Attachment, first 3 days of Degradation PLLA and PLGA) did exist but none of the students felt that this distracted considerably from the Module.

Laboratory instructors from Chemistry, Biology, Physics, Bioengineering, and Chemical Engineering are currently working on an assessment tool that will be used to evaluate the effectiveness of laboratory courses and also track a student's development in a laboratory setting from freshman year to senior year. When these materials are more developed, a more formal assessment of these two courses will be implemented on a routine basis.

Equipment and Facilities

For BIOE 342, the equipment and facilities required for a section size of 12 students are given below.

BIOE 342: Necessary	**BIOE 342: Nice To Have - Can Buy or Borrow**
• 6 Laminar flow hoods • 1-2 Centrifuges • 4-6 Inverted microscopes • 2-3 Fluorescence attachments for microscopes • Hemocytometers • 20-30 Micropipettes of various sizes • 12 Pipet-aids • Over-under incubator (for students' cells) • Refrigerator • 1-2 Water baths • pH meter • Balances	• 3 Coulter Counters • Water purification (deionizer) • Single incubator (for instructor's cells) • 1-2 Spectrophotometers

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Also required are disposable tissue culture supplies that include flasks, well plates, pipets, micropipette tips, etc. Common use reagents such as media, serum, PBS, and antibiotics are required. Finally, specialty reagents such as MTT, Live/Dead reagents, anti-PCNA, etc. are necessary.

The start-up costs, including all "Necessary" and "Nice To Have" items (except laminar flow hoods) and all supplies and reagents were $145,000. The major costs (~$100,000) were the over-under incubator ($7,000), single incubator ($4,000), 3 Coulter Counters ($37,000), 6 Nikon inverted microscopes ($25,000), 2 fluorescence attachments ($10,000), 2 centrifuges ($8,500), and the water purification system ($4,000). Yearly supplies and expenses for 50 students are estimated at $7,000-$8,000.

In addition to the equipment and facilities above, the following equipment is needed for implementation of the Tissue Engineering (TE) Module.

BIOE 441 TE Module: Necessary	**BIOE 441 TE Module: Nice To Have - Can Buy or Borrow**
• 2 Chemical fume hoods • Polymer synthesis glassware and vacuum system • GPC with autosampler • Vacuum oven and pump	• DSC • FTIR

Again, disposable tissue culture supplies are necessary. Common use reagents such as media, serum, PBS, and antibiotics are required. PLLA, PLGA, L-lactide monomer, and bulk reagents such as chloroform and methanol are necessary.

The start-up costs, including all "Necessary" items (except chemical fume hoods) and all supplies, reagents, and chemicals were $40,000. (This cost assumes equipment and facilities are available from BIOE 342.) The major cost was the GPC with autosampler ($30,000). Yearly supplies and expenses for 50 students are estimated at $3,000-$4,000.

Dissemination of Course Materials

The author is willing to provide copies of the protocols to other instructors upon request. Laboratory manuals will be uploaded onto the Web in 2002 or 2003.

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Appendix 1 - Schedule for BIOE 342, Lab Module in Tissue Culture

	Introductory Material	**HDF Cell Survival and Function**	**GFP Transfection Assay**
Session 1	• Micropipette • Cell Visualization • Sterile technique • Contamination • pH and color		
Session 2	• Cell concentration • Sterile technique		
Session 3	• Sterile technique	• Fn attachment • MTT prep • Live/dead staining prep	
Session 4		• MTT assay • Live/dead staining	
Session 5		• Attachment time • PCNA staining prep	
Session 6		• Proliferation • PCNA staining	
Session 7		• Proliferation	• GFP expt
Session 8		• Proliferation	• GFP expt
Session 9			• GFP expt
Session 10			• GFP expt

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Appendix 2 - Schedule for the Tissue Engineering Module in BIOE 441

	Monday (or Tuesday)	**Wednesday (or Thursday)**
Sessions 1, 2	• Prepare PLLA, PLGA films for degradation study • Run GPC on commercial PLLA and PLGA	• Prepare PLLA, PLGA films for degradation study • Run DSC and FTIR on commercial PLLA, PLGA
Sessions 3, 4	• Start PLLA, PLGA degradation study • PLLA synthesis	• PLLA synthesis • Degradation study maintenance
Sessions 5, 6	• Run GPC on synthesized PLLA • Prepare PLLA films for cell studies • Receive flask of HDF cells • Degradation study maintenance	• Begin cell assays • Degradation study maintenance
Sessions 7, 8	• Cell assays • Degradation study maintenance	• Cell assays • Degradation study maintenance
Sessions 9, 10	• Cell assays • Degradation study maintenance	• Cell assays • Degradation study maintenance
Sessions 11, 12	• Cell assays • Degradation study maintenance	• Run GPC on all degradation samples

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