AC 2012-4309: DEVELOPMENT AND ASSESSMENT OF A TEXTBOOK FOR TISSUE ENGINEERING LAB INSTRUCTION

Dr. Melissa Kurtis Micou, University of California, San Diego

Melissa Kurtis Micou is a lecturer in the Department of Bioengineering at the University of California, San Diego. She has taught tissue engineering lecture and lab courses to undergraduate students for the past seven years.

Dawn M. Kilkenny Ph.D., University of Toronto

Dawn Kilkenny earned her Ph.D. in physiology from the University of Western Ontario, Canada, and trained as a postdoctoral fellow in Immunology at Vanderbilt University, Nashville, Tenn. She subsequently worked for four years as a Senior Research Specialist at the Vanderbilt Cell Imaging Resource (CISR) microscope facility, and is currently an Assistant Professor at the Institute of Biomaterials and Biomedical Engineering (IBBME), University of Toronto. She is also the Academic Advisor to the IBBME Undergraduate Teaching Laboratory.

Development and Assessment of a Textbook for Tissue Engineering Lab Instruction

Abstract

Over the past decade, there has been a tremendous increase in the number of biomedical engineering/bioengineering (BME/BE) programs offering lecture courses in tissue engineering (TE), yet very few offer a lab component or separate lab course. Given that engineering is an applied field, the benefits of hands-on lab experience are clear. A new textbook entitled *A Laboratory Course in Tissue Engineering* will be published by Taylor Francis and CRC Press in summer 2012. The lab manual is appropriate for upper-division undergraduates or graduate students without prior hands-on TE experience, the content and structure are intended to facilitate development of new TE lab courses, and an instructor's manual is available. The experiments within the book are based on both classic TE experiments and modern TE techniques and emphasize the importance of engineering analysis, mathematical modeling, and statistical design of experiments. All of the experiments have been extensively tested and refined to improve the likelihood of successful data collection. Seven representative labs were formally assessed during the fall 2011 academic quarter at the University of California, San Diego. Results from an anonymous survey conducted at the end of the quarter indicate that learning outcomes were achieved and that students found the experiments both enjoyable and challenging. *A Laboratory Course in Tissue Engineering* provides a convenient source of instructional materials and, to our knowledge, will be the first commercially available lab manual for TE instruction.

Introduction

During the past decade there has been a tremendous increase in the number of biomedical BME/BE programs offering lecture courses in TE. An online search of current BME/BE curricula reveals that nearly seventy percent of ABET accredited BME/BE programs offer a TE lecture course, yet only roughly fifteen percent offer a lab component or separate lab course. The benefits of hands-on experience in this applied and interdisciplinary field are evident, yet very few programs offer associated lab courses. The small number of existing TE lab courses may be attributed, in part, to the relatively recent emergence of the field and the lack of available instructional materials. To help meet this need, a new textbook entitled *A Laboratory Course in Tissue Engineering* has been written and will be published as a paperback book by CRC Press/Taylor Francis in summer 2012. The book is approximately two hundred and fifty pages and contains over seventy original figures.

Overview of the Textbook

The lab manual is appropriate for upper-division undergraduate students with knowledge of engineering fundamentals, cell biology, and statistics, or for graduate students who are new to the field of TE. The experiments included within the book are based on both classic experiments[1,5,7-8] and more recent TE advances[2-4,6]. This collection of experiments was assembled to encompass a set of widely applicable techniques: cell culture, microscopy, histology, immunohistochemistry, mechanical testing, soft lithography, and common biochemical assays. In addition to teaching these specific techniques, the experiments emphasize engineering analysis, mathematical and computer modeling, and statistical experimental design.

The investment required to establish an instructional tissue engineering lab facility is substantial. To maximize the return on investment, experiments make extensive use of a shared set of equipment (Table 1). Due to the interdisciplinary nature of TE, it is possible to use this shared set of equipment to conduct experiments that reinforce a wide range of engineering and life science concepts that are covered in typical BME/BE programs. Supplies and consumables necessary for routine TE lab work are used throughout the book and should be made available to students (Table 1). Within each chapter, a list of additional *specialized* equipment, reagents, and supplies necessary for that particular experiment is provided. Care was taken to minimize the cost of reagents and consumable supplies. Suggested suppliers and product numbers for equipment and materials are provided in the instructor's manual along with other resources to help plan a new course.

General-Purpose Equipment & Supplies	**General-Purpose Consumables**
Analytical Balance	Serological Pipettes*
Glassware*	Pasteur Pipettes*
Small Instruments* (Forceps, Scalpels, Spatulas, etc.)	Pipette Tips*
Vortex Mixer	Weigh Boats/Weigh Paper
Magnetic Stir Plate with Stir Bars*	Tissue Culture Vessels*
Orbital Shaker	Conical Centrifuge Tubes*
Water Purification System	Microcentrifuge Tubes* and Vials*
Pipet-Aids	Autoclave Supplies (Bags, Tape, etc.)
Micropipettes	Filters for Liquid Sterilization*
pH Meter	Syringes* and Needles*
Centrifuge	Scalpel Blades* with Handles
4^oC Refrigerator	
-20^oC Freezer	
-80^oC Freezer and/or Liquid Nitrogen Storage	
Biosafety Cabinet (BSC)	
Vacuum Source for Liquid Aspiration	
CO_2 Incubator	
Autoclave	
Inverted Microscope with Image Capture	
Hemacytometer	

Table 1. General-purpose equipment, supplies, and consumables required for the experiments within the lab manual. *A sterile supply of these items should be readily available.

The content and structure of this textbook are intended to facilitate development of new TE lab courses. Fifteen standalone experiments provide significantly more than a semester's worth of activities, allowing instructors to customize their course. Most experiments conform to schedules commonly used in undergraduate lab courses (i.e., 2-4 hour sessions that can be offered once or twice a week). For added flexibility, experiments are broken up into as many different sessions as possible. Session durations are estimated based on the authors' experience with undergraduate students. Suggestions for shortening selected sessions with additional preparation by the instructor or teaching assistants are provided in the instructor's manual. Table 2 summarizes the topic of each experiment as well as the suggested schedule and specialized equipment that is required.

It is important for engineering students to develop strong communication skills to effectively document and disseminate their work. The "Data Analysis and Reporting" section within each chapter provides a framework for succinctly documenting the key results from each experiment. Additionally, Chapter 19 provides general guidelines for reporting results in the form of a technical report or journal article, extended abstract, abstract, or technical poster. Specific reporting formats are not prescribed within individual chapters. As such, the instructor can assign a variety of reporting formats throughout the semester, regardless of which experiments are conducted. The recommended structure for technical reports, journal articles, extended abstracts, and technical posters includes Abstract, Introduction, Materials and Methods, Results, Discussion, References, and Acknowledgments. A general description of the content in each section is provided. The instructor may provide more detailed reporting instructions by referring students to author guidelines published online for individual journals or conferences.

Assessment

All the experiments within the book have been extensively tested and refined. With the exception of experiments in Chapters 4, 7 and 8, this testing has occurred within the context of undergraduate lab courses at the University of California, San Diego or the University of Toronto. Individual students have validated the decellularization (Chapter 7) and adhesion (Chapter 8) experiments, but not in a formal course environment. The chondrocyte isolation procedure in Chapter 4 is routine and, although time limitations of an instructional lab format prevent optimal tissue digestion, a healthy population of cells should be readily obtainable from viable tissue. Some of the experiments are sophisticated and technically challenging however the protocols have been designed to create a high probability of successful data collection. Given the nature of TE experiments, student-collected data often exhibits significant variability and/or differs from the expected results. This provides an excellent learning opportunity by introducing students to the difficulties associated with analyzing authentic data and challenging them to critically analyze all elements of the protocol to determine why their results are not as expected. It also encourages discussion of experimental design and statistical power.

Chapter: Experiment Title	Schedule [hrs. unless indicated]	Specialized Equipment
Ch 4: Isolation of Primary Chondrocytes from Bovine Articular Cartilage	4	Fluorescence Microscope
Ch 5: Measuring and Modeling Growth of a Cell Population	3; *(1 week*);* 3	Fluorometer
Ch 6: Purification of a Cell Population Using Magnetic Cell Sorting	3	DynaMag^{TM}, Platform Rocker, Fluorescence Microscope
Ch 7: Decellularized Matrices for Tissue Engineering	2; (~48); 2; 2/2	Platform Rocker, Cryostat
Ch 8: Effect of Plating Density on Cell Adhesion to Varied Culture Matrices	4; 4	Orbital Shaker, Microplate Reader
Ch 9: Dynamic vs. Static Seeding of Cells onto Biomaterial Scaffolds	2/2; (~24-48); *1;* 3	Spinner Flask, Fluorometer
Ch 10: Cell Patterning Using Microcontact Printing	2; 2; 3; 2	Vacuum Chamber, House Air, Fluorescence Microscope
Ch 11: Measuring & Modeling the Motility of a Cell Population Using an Under Agarose Assay	2/2; (~24-48); 1	-
Ch 12: Characterizing Matrix Remodeling Through Collagen Gel Contraction	3; 2	Fluorescence Microscope
Ch 13: Effect of Substrate Stiffness on Cell Differentiation	2; 1; 2; 1; 3; 2	Fume Hood, Vacuum Chamber, UV Lamp, Fluorescence Microscope
Ch 14: Effect of Culture Configuration (2D vs. 3D) on Cell Phenotype	3; (1 week); 2; 1/1	Fluorometer, Microplate Reader
Ch 15: Combining *in silico* and *in vitro* Techniques to Engineer Pluripotent Stem Cell Fate	3; varies; 3; 3; 4; (~24); 3; (~24); 4	Spin Coater, UV Curer, Orbital Shaker, Programmable Hot Plate, Humidifier w/Chamber, N_2 Gun, Fluorescence Microscope
Ch 16: The Fahraeus-Lindqvist Effect: Using Microchannels to Observe Cell Flow	4	Custom Fabricated Microfluidic Device
Ch 17: Examining Single Cell Mechanics Using a Microfluidic Micropipette Aspiration System	4	Custom Fabricated Microfluidic Device
Ch 18: Contribution of Microarchitecture to Bone Strength	2; varies	Dremel Tool, Material Testing Machine

Table 2. Overview of individual experiments, schedules, and specialized equipment required. Session durations that appear with a slash (e.g., 2/2) may be combined. Unless indicated in parenthesis, time between consecutive sessions is flexible. Italics indicate that the instructor may perform interim steps without loss of continuity. *With periodic sample collection (requires ~15 min per day) by students or instructors.

Seven representative labs from the textbook were formally assessed during the fall 2011 academic quarter at the University of California, San Diego. Specifically, seventy-five senior undergraduates enrolled in *BENG162: Biotechnology Lab* worked in teams to complete four experiments from the textbook (a random sub-set of the seven tested) plus two traditional biotechnology experiments that have been included in the course for many years. The TE experiments tested were Cell Sorting (Chapter 6), Cell Seeding (Chapter 9), Cell Patterning (Chapter 10), Modeling Cell Migration (Chapter 11), Matrix Remodeling (Chapter 12), Cell Differentiation (Chapter 13), and 2-D vs. 3-D Culture (Chapter 14). The traditional biotechnology experiments involved separating a protein mixture using high-performance liquid chromatography (HPLC) and measuring oxygen transport across the gas-media interface of a stirred-tank bioreactor. An anonymous survey conducted at the end of the quarter was used to indirectly measure achievement of specific learning outcomes and to compare student attitudes about the TE and traditional biotechnology experiments. In the latter case, the HPLC and Bioreactor experiments served as convenient controls for comparison against the TE experiments. Although this type of data collection is exempt from institutional review board oversight, students provided written consent to participate in this assessment.

Students were asked to indicate their agreement (on a Likert scale of 1 to 5) with statements related to the knowledge and skills they gained from the course (Table 3). Although it was impractical to attempt to differentiate between the contributions from the TE labs compared to the HPLC and Bioreactor labs, the overall positive results are encouraging.

BENG 162….	**mean±SD**
…provided opportunities to *apply biology, math, and engineering skills* gained from previous coursework.	4.3 ± 0.5
…increased my *ability to design an experiment.*	3.6 ± 0.8
…taught me useful lab skills, which have increased my *ability to collect data.*	4.2 ± 0.8
…increased my *ability to analyze experimental data*, including statistically.	4.0 ± 0.7
…increase my *ability to function on a team* to complete tasks.	3.9 ± 0.9
…increased my *knowledge of contemporary issues* or the current state of the field.	4.1 ± 0.8
…increased my *ability to synthesize current and relevant information.*	3.8 ± 0.7
…increased my *ability to communicate effectively* via reports, posters, abstracts, etc.	4.2 ± 0.8
…increased my *competency as a researcher.*	4.0 ± 0.7

Table 3. Average student response to questions related to achievement of specific learning outcomes (n=74).

Overall, the students reported that the course was more enjoyable (3.9±0.8) and more challenging (3.8±0.8) than other lab classes they had taken. The required lab courses completed by students in this major are general chemistry lab, physics lab, and molecular bioengineering techniques. The results are surprising because course enrollment more than doubled compared to previous years due to an unrelated department policy change. The large class size necessitated the inclusion of five students in each lab group and an unconventional class schedule. Despite these substantial logistical challenges, the class and experiments were generally successful.

In the survey, students were prompted to indicate the three most enjoyable and three most intellectually challenging experiments and to provide a brief explanation for their ranking. To quantify the responses, points were assigned to the highest (3 pts), second highest (2 pts), and third highest (1 pt) rated experiment in each category. In general, students indicated that the seven TE experiments were more enjoyable (1.0-1.9) than the HPLC (0.9±1.2) or the bioreactor lab (0.7±1.2), although some of these differences were small. The most enjoyable TE labs were cell sorting (1.9±1.4), cell differentiation (1.7±1.2), and remodeling (1.6±1.1). Comments indicated that students felt the most enjoyable labs were "more hands on", "challenging", and produced results that were "easy to see". In contrast, the HPLC and bioreactor labs involved a "set, forget, and collect" approach. Enjoyment of labs did not directly correlate with the level of difficulty. Students identified the most intellectually challenging labs as cell migration (2.1±0.9), bioreactor (1.8±1.2), and cell patterning (1.4±1.3). HPLC was among the easiest (0.6±0.8) and least enjoyable. Representative comments indicated that level of difficulty was related to data analysis, statistics, and experiments "not going as planned". The cell migration and bioreactor labs are also the most mathematically intense.

Students rated the quality of course materials including the lab guides (3.7±0.9) and pre- and post-lab questions (3.7±0.9) favorably, however, indicated that there was room for improvement. Suggestions for specific points of clarification were collected using an online discussion forum and used to revise the textbook. As described above, the final chapter in the textbook provides guidelines for reporting experimental findings as a technical report, extended abstract, and technical poster. These formats were also used in BENG 162. Nearly all students commented on the variability of the reporting format and most enjoyed this aspect of the course (4.3±1.0). As a consequence of the favorable student responses, *A Laboratory Course in Tissue Engineering* will be a recommended textbook for BENG 162 and BME340: Biomedical Engineering Technology and Application, a third year undergraduate course at the University of Toronto.

Conclusions

A Laboratory Course in Tissue Engineering provides a set of cohesive instructional materials to enhance existing TE lab courses or allow creation of new lab sessions to support existing TE curricula. To our knowledge, this is the first commercially available lab manual for TE instruction. Preparation of this textbook was prompted by the numerous requests the authors and contributors receive for the protocols used in their courses, a desire to initiate a network for sharing instructional protocols, and the hope that more lab courses in TE will be offered to students in the near future.

Each experiment within the textbook is a standalone component so that instructors may select the combination of experiments that best fit their curricula, available resources, and class schedule. The experiments have been extensively validated and cover a wide-range of technical aspects relevant to support hands-on pedagogy in this emerging scientific field. A typical course might begin with students completing step-by-step exercises that introduce them to sterile technique, media preparation, thawing cryopreserved cells, passaging cells, and determining cell density using a hemacytometer. These exercises are provided in Chapter 3 of the text. Next, students may work in groups to conduct a few experiments that provide ample opportunity to reinforce these essential skills, for example, cell isolation (Chapter 4), expansion of a cell population (Chapter 5), and cell separation (Chapter 6). Together, these activities should take approximately five weeks to complete. For the rest of the semester, the instructor may select several of the more sophisticated experiments that complement the course objectives. To facilitate this process, specific learning objectives are provided within each chapter (e.g., *fit experimental data to a mathematical model of cell migration to extract a motility coefficient*). In a graduate course, it may be preferable to skip Chapters 4-6 in favor of the more sophisticated experiments. If the specialized equipment required for one or more of the experiments is limited, several protocols may be run simultaneously with student groups rotating through each experiment. For example, assume each section of the class meets twice per week (Mon/Wed or Tue/Thurs) and the lab can accommodate teams of three working simultaneously on five different experiments. By running sections in both the morning and afternoon and rotating teams through the experiments, it is possible to train 60 students with only one set-up per experiment.

TE is an exciting, dynamic, and interdisciplinary field. Offering a lab course in TE provides BE/BME departments with an opportunity to train their students in modern laboratory techniques while integrating and reinforcing life science and fundamental engineering concepts.

Acknowledgments

We are extremely grateful for the contributions of our colleagues to this book, especially Karen Christman, Adam Engler, Noah Goshi, Craig Simmons, Wujing Xian, and Peter Zandstra.

References

1. Bell, E., B. Ivarsson, and C. Merrill. 1979. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proc Natl Acad Sci U S A* 76 (3):1274-8.
2. Davey, R. E., K. Onishi, A. Mahdavi, and P. W. Zandstra. 2007. LIF-mediated control of embryonic stem cell self-renewal emerges due to an autoregulatory loop. *FASEB J* 21 (9):2020-32.
3. DeQuach, J. A., V. Mezzano, A. Miglani, S. Lange, G. M. Keller, F. Sheikh, and K. L. Christman. 2010. Simple and high yielding method for preparing tissue specific extracellular matrix coatings for cell culture. *PloS one* 5 (9):e13039.
4. Engler, A. J., S. Sen, H. L. Sweeney, and D. E. Discher. 2006. Matrix elasticity directs stem cell lineage specification. *Cell* 126 (4):677-89.
5. Lauffenburger, D., C. Rothman, and S. H. Zigmond. 1983. Measurement of leukocyte motility and chemotaxis parameters with a linear under-agarose migration assay. *Journal of immunology* 131 (2):940-7.
6. McDevitt, T. C., J. C. Angello, M. L. Whitney, H. Reinecke, S. D. Hauschka, C. E. Murry, and P. S. Stayton. 2002. In vitro generation of differentiated cardiac myofibers on micropatterned laminin surfaces. *J Biomed Mater Res* 60 (3):472-9.
7. Theret, D. P., M. J. Levesque, M. Sato, R. M. Nerem, and L. T. Wheeler. 1988. The application of a homogeneous half-space model in the analysis of endothelial cell micropipette measurements. *J Biomech Eng* 110 (3):190-9.
8. Vunjak-Novakovic, G., B. Obradovic, I. Martin, P. M. Bursac, R. Langer, and L. E. Freed. 1998. Dynamic cell seeding of polymer scaffolds for cartilage tissue engineering. *Biotechnol Prog* 14 (2):193-202.