**Tissue Engineering Laboratory**

**BE 5365 (BIOL 4365), Summer 2014 (Drafted)**

Tuesday/Thursday 3:30-5:20pm

ERB 273

**Instructor:** Dr. Liping Tang

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**Course Description**: Introduction to Laboratory techniques commonly used for culturing, growing, and analyzing cells and tissues.

**Course Learning Goals/Objectives:** Students will learn the necessary skills required for maintaining and analyzing cells in culture. Students will develop laboratory techniques related to cell assays and cell staining. Students will be introduced to concepts of designing in vitro tissue engineering products.

**Textbooks:** Peer-reviewed journals and laboratory protocols will be used for this course and when applicable made available to students through the course folder.

Suggested Texts: For cell culture review

* *Culture of Animal Cells – A Manual of Basic Technique 4th edition, by Ian Freshney, 2000*
* *UTA library call #QH585.2 .F74 2000 or* [http://discover.uta.edu/?itemid=|uta-cat|1047659](http://discover.uta.edu/?itemid=%7Cuta-cat%7C1047659)

Tentative Syllabus/ Laboratory Schedule:

Jun 5: Syllabus, Class introduction, group assignments.

**Lecture:** Course overview and introduction

**Handouts:** Equipment use / ftp class files

**Lab:** Equipment overview / use and operation

Jun 7: Serial dilutions

**Lecture:** Use of spectrophotometer to measure absorbance, applications in tissue engineering

**Lab:** Use of micro liter pipettes to make serial dilutions

**Practical Quiz 1:** comparison to TA standards using spectrophotometer

Jun 12: Basics of a presentation/ Report #1 / Introduction to H&E Staining

**Lecture:** Outlining a presentation/ finding research papers

**Discussion:** Overview of Report #1 / Overview of H&E staining

**Lab:** H&E staining of biomaterial implant

Jun 14: Identifying organs with H&E stain

**Lecture:** H&E stain to identify organs as well as characteristics of the foreign body response to implants

**Demo:** Wax sectioning of tissue samples

**Lab:** H&E staining of organs

Jun 19: Dr Ashwin Nair - salt leached scaffold fabrication

**Lecture:** scaffold basics

**Lab:** PLGA salt leached scaffold fabrication

**Homework 1:** Salt leaching process with tap water

Jun 21: Dr Ashwin Nair - scaffold characterization

**Lecture:** porosity of scaffolds

**Demo:** testing of salt leaching water with Silver Nitrate

**Lab:** Ethanol displacement method to calculate porosity

**Note:** Be sure to place one scaffold section in OCT

**Homework 2:** Calculations for scaffold porosity

Jun 26: Quiz on H&E staining / Image J and Photoshop

**Demo:** Image J area calculations and porosity / Use of Photoshop

**Practical Quiz 2:** H&E staining

**Lab:** observe scaffold cross sections and H&E stains on microscope

**Homework 3:** Image processing. Given image use Photoshop or Image J to adjust as found in published works

Jun 28: Masson Trichrome overview

**Lecture:** Trichrome staining to identify characteristics of the foreign body response to implants

**Lab:** Collagen staining

Jul 3: Toluidine Blue overview

**Lecture:** Toluidine Blue staining to identify mast cells

**Lab:** Toluidine blue staining / Observe collagen stain/ pictures

**Demo:** Foreign body response calculations using Image J

**Homework 4:** Foreign body response calculations

Jul 5: Practical Quiz on staining techniques

**Quiz 3:** Masson Trichrome and Toluidine blue staining

**Lab:** Retake images as needed for report

Jul 10: **Group Presentations (Midterm)**

**Report #1 due**

Jul 12: Introduction to cells and cell culture I

**Lecture** Introduction to cells, the 3T3 fibroblast

**Video:** Basic cell culture procedure, Aseptic technique in a culture hood

**Lab:** Preparing a cell hood for cell culture, making “complete” media, media change in a culture flask

Jul 17:Introduction to cells and cell culture II, cell viability

**Lecture:** Observing cells, morphology and cellular density

**Lab:** Observing cell morphology / View field area calculations

Use of Hemocytometer to calculate cell viability with trypan blue

**Homework 5:** Area calculations and cell viability

Jul 19: Introduction to cells and cell culture III, subculture

**Lecture/ video:** subculture and trypsinization

**Lab:** Subculture of cells with trypsin

**Discussion:** Report #2 outline

Jul 24: Subculture and seeding density / Quiz

**Lecture:** Online searching for cell lines ATCC

**Lab/ practical Quiz 4:** Subculture of cells, seeding of well plate at specified density

**Homework 6:** Image J cell counting

Jul 26: Growth rate of cells / Alamar Blue Assay

**Lecture:** Alamar Blue and cell titer assays for cell quantification

**Lab:** Estimation of growth rate with Alamar Blue

**Demo:** standard curve / viability assay

Jul 31: Primary culture, subculture

**Lecture:** Overview of tissue digestion and primary culture

**Video:** harvesting neonate tissue

**Lab:** Estimation of growth rate with Alamar Blue

**Lab:** Bone marrow cell extraction, primary culture

Aug 2: Primary Culture / Quiz

**Lab:** analysis of primary cell culture, cell density

**Practical Quiz 5:** Primary culture and growth rate

Aug 7: **Group Presentations (final)**

Aug 9: **Final Reports due**

**Aug 9th Last Day of Classes**

**Overview of Course components**

**Homework** will consist of short 1 or 2 page write-ups of a given topic. Homework is directly related to experiments and/or projects. It is often required that 2-4 peer -reviewed journals are listed as references or other website or text book sources.

**Quizzes** will be short answer format and are based on the assignments or topics covered in class.

**Reports:** Two reports, which will be written individually, will consist of a journal style presentation of the topics assigned. Reports are due at the start of class on the day specified in the syllabus. **NO LATE REPORTS WILL BE ACCCEPTED!**  More detail on format and requirements will be given prior to the report due dates.

**Presentations:** Each report will be accompanied by a presentation. Presentations will be done in groups. Presentations are due at the start of class on the day your group is assigned to present. **NO LATE PRESENTATIONS WILL BE ACCCEPTED!** More detail on format and requirements will be given prior to the report due dates.

**Course Evaluation & Final Grade:**

*15% Homework/Quizzes/Labs + 15% Presentation 1 + 15% Report 1 + 15% Presentation 2 + 15% Report 2 + 25% Attendance and Participation*

**Americans With Disabilities Act.** The University of Texas at Arlington is on record as being committed to both the spirit and letter of federal equal opportunity legislation; reference Public Law 93112 -- The Rehabilitation Act of 1973 as amended. With the passage of new federal legislation entitled Americans With Disabilities Act - (ADA), pursuant to section 504 of The Rehabilitation Act, there is renewed focus on providing this population with the same opportunities enjoyed by all citizens.

As a faculty member, I am required by law to provide **"reasonable accommodation"** to students with disabilities, so as not to discriminate on the basis of that disability. Student responsibility primarily rests with **informing faculty at the beginning of the semester and in providing authorized documentation through designated administrative channels.**

**Academic Dishonesty.** It is the philosophy of The University of Texas at Arlington that academic dishonesty is a completely unacceptable mode of conduct and will not be tolerated in any form. All persons involved in academic dishonesty will be disciplined in accordance with University regulations and procedures. Discipline may include suspension or expulsion from the University.

"Scholastic dishonesty includes but is not limited to cheating, plagiarism, collusion, the submission for credit of any work or materials that are attributable in whole or in part to another person, taking an examination for another person, any act designed to give unfair advantage to a student or the attempt to commit such acts." (Regents' Rules and Regulations, Part One, Chapter VI, Section 3, Subsection 3.2, Subdivision 3.22)