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Experimental methods in tissue engineering: An integrated approach to theory, design, and analysis

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Abstract

Tissue engineering involves the design, construction and characterization of tissue constructs to model tissue function or to be used as a regenerative medicine therapeutic. Often, in tissue engineering laboratory courses, much emphasis is given to biomaterial synthesis, biomechanics, and biotransport with little focus on quality assessment of tissue constructs. Thus, we developed a theory, design, and analysis (TDA) framework to provide undergraduate students with more practice in tissue characterization. The framework involves structuring a multi-week lab that integrates theoretical foundations, bioinstrumentation background, experimental design, and data analysis. The goal of the framework is to enhance lab-based learning by providing opportunities for students to incorporate multiple levels of Blooms Taxonomy. By consolidating these opportunities into a multi-week module, we hypothesized that students would experience more reinforcement and thus self-efficacy with these experimental methods. For this study, we focused on the development of a TDA module to measure apoptosis in tissue constructs using real-time, reverse transcription polymerase chain reaction (RT-PCR). Before deployment of this module, students were presented with a Likert survey (5-point scale with 1 being strongly disagree and 5 being strongly agree) to gauge their comfortability (as a measure of self-efficacy) with experimental techniques, experimental design, data analysis, and their ability to describe apoptotic mechanisms. Students then participated in a series of "wet" and "dry" lab exercises to promote TDA competency in tissue characterization by real-time RT-PCR. Afterwards, students completed a post-lab Likert survey to assess outcomes. Based on our analysis, students expressed enhanced self-efficacy in performing real-time RT-PCR (2.9 vs. 4.1, $p < 0.01$), analyzing gene-expression data (3.1 vs. 3.9, $p < 0.05$) and explaining the mechanisms of apoptosis (3.3 vs. 4.1, $p < 0.01$) after completing the TDA module. Given these results, we have expanded the use of TDA modules in this course to promote self-efficacy with other experimental methods used in tissue engineering including flow cytometry and rheometery.

Introduction

Tissue engineering involves the design, construction and characterization of tissue constructs to use as a therapeutic, diagnostic or disease model. In many college-level bioengineering programs across the United States, tissue engineering is embedded as a core course to support programmatic outcomes. Indeed, the dynamic rate of change within the field and the growing workforce needed to support these changes underscore the importance of such courses. Current frameworks of tissue engineering courses include those with and without a laboratory component. Unfortunately, for many courses, the laboratory exercises do not tie directly back to the concurrent lecture material. [1], [2] Additionally, many labs focus on foundational skills such as the synthesis of biomaterials, tissue biomechanics and biotransport with little focus on the characterization of tissue engineered constructs, an essential step to prototyping and ensuring engineered tissue quality. Thus, we sought to develop a fully integrated experience for students to promote self-efficacy in tissue characterization using a novel theory, design and analysis (TDA) framework.

TDA is based on similar high impact practice frameworks including the "how people learn" [3] "project-based learning" [4], [5] and "design-based learning" [6], [7] frameworks and uses three integrated modules to promote theoretical and technical competency. Importantly, the integration of pedagogical theory and practice is not novel in of itself [8], [9], though the integration to support technical skillsets that reinforce design and data analysis is a novel component to the TDA framework.

- **Theory**: Traditional lectures that take place in the classroom and the laboratory. During this module students are engaged around a critical topic in tissue characterization. Mechanistic background related to the underlying cellular processes is provided to aid students in understanding how such processes can affect engineered tissue quality. Students are then presented with a conceptual framework for analyzing the underlying cellular process.
- **Design**: Students review the standard workflow used to support investigations of the outlined cellular process. From there, students will design an experiment with stated hypothesis, controls, replicates and supporting calculations. These designs are submitted to the instructor for review before experiments are run. Once approved, students will run their experiments as designed.
- **Analysis**: Students are initially provided with sample data to gain insight into performing appropriate analysis of data collected from a given experimental workflow. Students will then apply these analytic tools to assess experimental outcomes.

After students have gone through the TDA experience, they submit a written report of their findings. As shown in Figure 1, the TDA framework integrates learning objectives across multiple levels of Blooms Taxonomy [10]. These levels build on each other due to the integrated nature of the topic, hands-on learning, and analytical tools discussed.

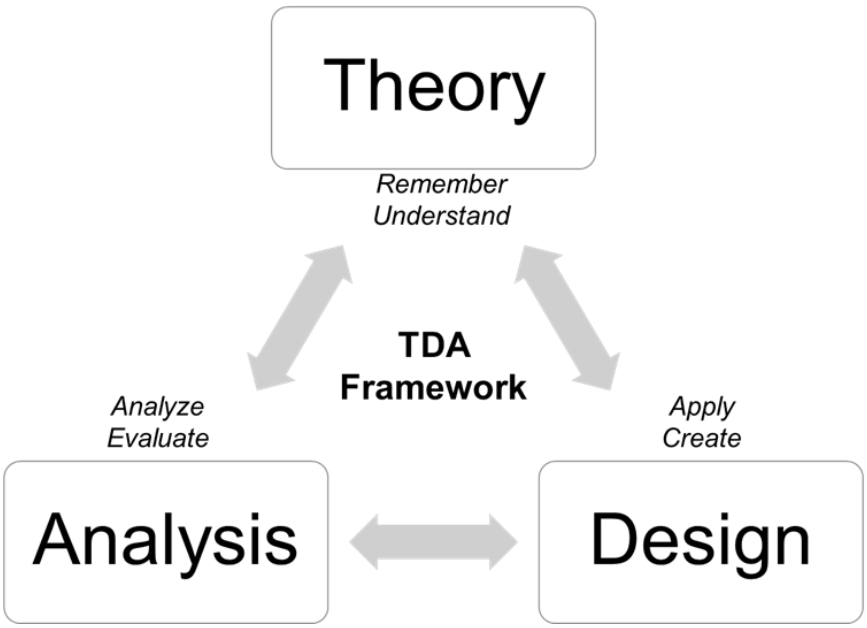

Figure 1: The integrated TDA framework with Blooms Taxonomy levels indicated (italics).

Materials and Methods

To demonstrate the effectiveness of the TDA framework in promoting learning, a two-week experience was developed around engineered tissue characterization to assess the cellular process of apoptosis using real-time reverse transcription polymerase chain reaction (RT-PCR). Components of this specific TDA experience included the following modules:

- Module 1 (Theory): Discussion of a canonical pathway of apoptosis that involves the regulation of two proteins: BAX and BCL-2. Students learn that the ratio of these two molecules can act as an indicator of apoptosis and that gene expression is one way of assessing the relative quantity of protein. During this module, students also discuss state of the art techniques used to isolate genetic material from cells and tissue and describe how RT-PCR is used to assess gene expression.
- Module 2 (Design and Experimentation): Discussion of sample workflows and reagents used to design and execute RT-PCR experiments. During this module students are engaged in a laboratory setting and are responsible for designing an experiment to assess BCL-2/BAX gene expression in control cells and cells exposed to a pro-apoptotic drug (hydrogen peroxide). From this design, students will need to execute experiments to isolate RNA, perform reverse transcription, and perform PCR with appropriate controls and replicates.
- Module 3 (Analysis): Discuss common analytical techniques to assess RT-PCR data. During this module, students will practice performing analysis using sample data before using the analytical tools to assess their own data. During this module, students are required to report on descriptive statistics, results of an appropriate statistical test, and make conclusions based on the analysis.

At the conclusion of the TDA experience, students were also required to submit a final report which was assessed using the following learning objectives:

1. Discuss the cellular mechanism of apoptosis including the role of the proteins BCL-2 and BAX in mediating this process.
2. Design experimental methods to assess gene expression using real-time RT-PCR.
3. Execute an experiment for real-time PCR by demonstrating the ability to isolate and quantify RNA, set up a reverse transcription reaction and perform PCR
4. Analyze real-time RT-PCR data using the delta-delta Ct method for control and apoptotic samples
5. Discuss conclusions of data based on calculated BCL-2/BAX ratios.
6. Communicate findings via a technical report.

Description of hands-on laboratory experiences: To isolate RNA, students used a standard workflow designed by Qiagen (Germantown, MD). Use of the Qiagen mRNeasy Kit allows for rapid isolation of pure messenger RNA. Students then used a Qubit device to measure the RNA concentration. Afterwards, students were responsible for calculating the volume needed for 100ng of RNA. This was then added to a reverse transcription assay mixture (Applied

Biosystems; Waltham, MA) and allowed to incubate for 1 hour at 37^{o}C followed by a 5-minute incubation at 95^{o}C to stop the reaction. Afterwards, students performed the appropriate calculations to determine the volumes needed to run duplicate control and apoptotic samples for the genes BCL-2 and BAX. Next, reagents from the PowerTrack SYBR Green kit (Applied Biosystems; Waltham, MA) were mixed, loaded into a 96 well plate and run using the Applied Biosystems QuantStudio Real-Time PCR system. Data was delivered to students in a Microsoft Excel worksheet and students could use any statistical package of their choosing to run the appropriate analysis.

Assessment of student self-efficacy: Prior to the start of the TDA experience, a pre-lab survey was used to collect information regarding student comfortability (as a measure of self-efficacy) [11] with applicable experimental techniques, data analysis and comprehension of the general process of cellular apoptosis. The survey was set on a 5-point level of agreement Likert Scale (with 1 being strongly disagree and 5 being strongly agree) and included the following statements:

- I am comfortable with the procedure to isolate RNA from cells
- I am familiar with real-time RT-PCR
- I am comfortable performing real-time RT-PCR
- I am comfortable analyzing data obtained from PCR experiments to determine levels of gene expression
- I am familiar with the processes and mechanisms involved in cellular apoptosis

After the TDA experience, students were presented with a post-lab survey that asked the same questions. Data is presented as the mean ± standard error and a two-tailed student t-test was used to measure statistical significance.

Student Background: Data was collected in the Spring 2022 semester from 13 juniors. Students were introduced to the TDA assignment during a required Biomaterials and Tissue Engineering course. Prior to this course, students had exposure to some of these techniques in a required sophomore-level Advance Molecular Biology course, though this course lacked elements of theory, design and analysis, the three major components of the TDA framework. Several students had also used RT-PCR as part of a required co-op experience in the semester prior to introduction to TDA.

Results

In general students reported increased self-efficacy in executing experimental methods, analyzing data and discussing mechanisms associated with apoptosis. As shown in Figure 2, students were generally just as familiar with real-time RT-PCR before the TDA experience compared to after ($p = 0.11$).

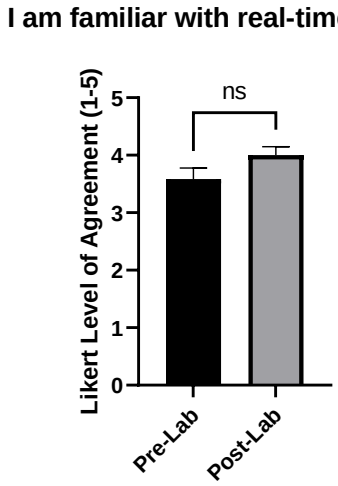

Figure 2: Self-reported level of agreement with the statement "I am familiar with real-time RT-PCR" before and after the TDA experience.

Despite being familiar with RT-PCR, students were generally less comfortable performing this technique before the TDA experience compared to after (Figure 3). In this case, we observed a statistically significant increase in self-reported self-efficacy ($p = 0.0008$) of more than one Likert point. Familiarity with RT-PCR is likely the result of being introduced to this technique in a required Advanced Molecular Biology course in their sophomore year.

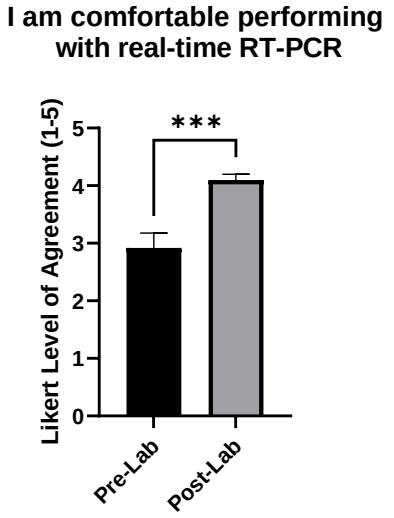

Figure 3: Self-reported level of agreement with the statement "I am comfortable performing real-time RT-PCR" before and after the TDA experience.

Students also reported being more comfortable analyzing data from PCR experiments after the TDA experience (Figure 4; $p = 0.045$), though there was no significant difference for RNA isolation procedures (Figure 5; $p = 0.07$).

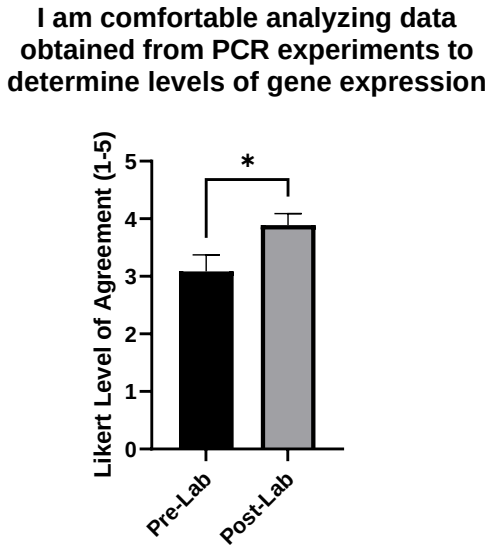

Figure 4: Self-reported level of agreement with the statement "I am comfortable analyzing data obtained from PCR experiments to determine levels of gene expression" before and after the TDA experience.

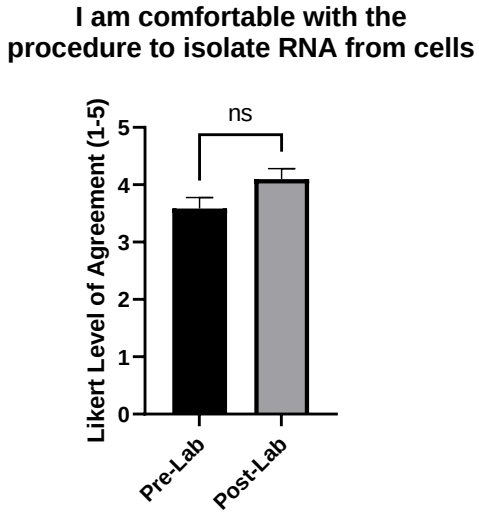

Figure 5: Self-reported level of agreement with the statement "I am comfortable with the procedure to isolate RNA from cells" before and after the TDA experience.

Lastly, students reported increased familiarity with the mechanisms of cellular apoptosis (Figure 6, $p = 0.0009$).

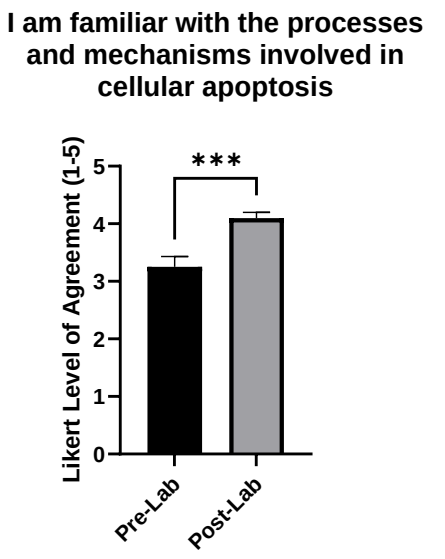

Figure 6: Self-reported level of agreement with the statement "I am familiar with the processes and mechanisms involved in cellular apoptosis" before and after the TDA experience

As part of the TDA experience, a final report was required to demonstrate competency in learning outcomes 1-6 as described above. As shown in Figure 7, students generally met expectations for all outcomes except for outcome 1 and 5 ("discuss the cellular mechanism of apoptosis including the role of proteins BCL-2 and BAX in mediating this process" and "Discuss conclusions of data based on calculated BCL-2/BAX ratios").

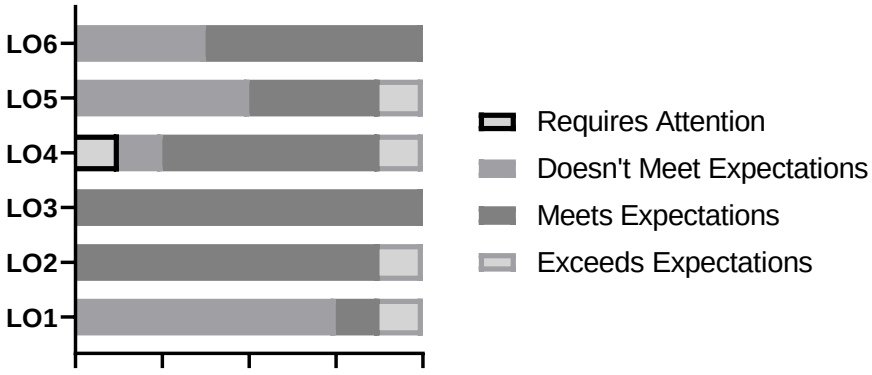

Figure 7: Assessment of learning outcomes associated with final reports

Discussion

Laboratory techniques in tissue engineering can provide valuable skillsets with real-world applicability. To support an often-overlooked aspect to the tissue engineering process (i.e.

quality assessment) a TDA framework was used to integrate multiple levels of learning around defined techniques in tissue characterization. The results of this study suggest that a TDA framework can promote self-efficacy with experimental techniques, data analysis and theoretical foundations. The integration of these attributes into a concise laboratory experience likely facilitated these results. Indeed, many other studies have demonstrated the importance of connecting lectures and labs in undergraduate education to enhance overall learning [2], [9], [12].

When reviewing the results of this study, two non-significant outcomes were identified. This included student familiarity with RT-PCR (Figure 2) and self-efficacy isolating RNA from biological samples (Figure 5). These results are likely tied to the learning outcomes associated with prerequisite courses for Biomaterials and Tissue Engineering. These prerequisites included experiences in advanced molecular biology techniques including PCR and isolation of DNA and RNA. This common thread facilitated reinforcement of learning outcomes and retention of valuable skillsets in engineered tissue characterization. Indeed, as shown above, students reported a significantly higher level of comfort performing RT-PCR (Figure 3) and analyzing data from these experiments (Figure 4). Due to the integrated nature of the TDA framework, concepts discussed in lecture were concurrently linked to hands-on, laboratory experiences to further reinforce learning outcomes. For this module, we were able to successfully link the mechanisms of BCL-2/BAX mediated apoptosis to relevant experimental procedures that could be used to directly measure BCL-2 and BAX gene expression. As a result, students reported a higher level of familiarity with mechanisms involved in cellular apoptosis (Figure 6).

In addition to enhanced self-efficacy in performing RT-PCR and analysis of associated data, students generally demonstrated competency in many of the learning outcomes attached to the TDA experience as assessed via a final report (Figure 7). This includes the ability to design and execute RT-PCR experiments, effectively communicate technical information, and analyze RT-PCR data. Students did, however, have more difficulty interpreting statistical analysis of data and describing the specific roles of BCL-2 and BAX in apoptosis. In reviewing the final reports, many students focused more on the general phenotypic consequences of apoptosis instead of the molecular mechanisms involving BCL-2 and BAX. Despite this, most students correctly referenced the ratio of BCL-2 to BAX as an important output in defining apoptosis. Additionally, although students had taken a biostatistics course prior to this experience, it was clear that competency in the use and interpretation of hypothesis tests was lacking. Informal discussions with students confirmed this observation and allowed for the opportunity to introduce more statistics into the curriculum of the Biological Engineering program in subsequent courses.

Despite these results, there are some limitations that will be considered and incorporated as we build and study other TDA experiences. For instance, a pre-lab/post-lab comparison doesn't fully demonstrate the effectiveness of TDA in relation to standard practice. Thus, we will integrate a "true" control to better understand the impact of TDA in the teaching and learning environment. Additionally, this short-term study did not assess longitudinal retention of skills in subsequent courses. If self-efficacy is what we are truly trying to achieve, longer term studies should be incorporated to fully assess reinforcement and impact. [12]

Conclusion

The TDA framework provides an integrated experience for students to link theory to hands-on practice. Given the need to develop a workforce with practical skillsets in biological engineering, the TDA framework may provide an effective means to promote technical self-efficacy with appropriate skillsets in the design and analysis of complex experiments.

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