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*Dr. Barthell transitioned from his role as Dean of the College of Mathematics and Science to Provost and Vice President of Academic Affairs while this journal was in the final stages of preparation. It was initiated under his guidance and would not have been possible without his early work.
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Preface

John Barthell¹, and Charlotte Simmons²

¹Provost and Vice President of Academic Affairs and ²Dean of the College of Mathematics and Science

“There was a time not so long ago when the great faculty divide was between faculty who performed research and faculty who did not. Now, however, with most faculty engaged in research, the new line of demarcation is instead between faculty who engage students in their research and those who do not.” - Malachowski (2006)

This inaugural volume of the Journal of Undergraduate Research (JUGR) is the outcome of the long and multifaceted history of the College of Mathematics and Science (CMS). That history manifests itself daily in the dynamic and creative milieu of seven departmental communities that commit themselves to a common goal: helping students learn. This college, however, has long excelled in facilitating learning in a way that relies on mentorship and the many benefits accrued from that approach to learning. Perhaps at no other time in our history is that approach more in need than it is today.

At a time when economic projections indicate that approximately one million more STEM professionals than the U.S. will produce at the current rate will be needed to “retain its historical preeminence in science and technology,” fewer than 40% of undergraduates declaring a STEM major nationwide complete a STEM degree (PCAST, 2012). As a Predominantly Undergraduate Institution (PUI) where faculty and administrative efforts are concentrated on creating a student-centered culture that assists students in achieving their educational objectives, the University of Central Oklahoma (UCO) is a logical setting for projects focused on increasing STEM retention and graduation rates. Referred to as “Transformative Learning” on our campus, UCO employs a learning-centered approach proposed by Elgren (2004) that fosters scientific inquiry and encourages faculty and student involvement in research activities promoting student learning, faculty development, and the development of new knowledge (Barthell, 2012b). Indeed, UCO is committed to experiential learning and is recognized nationally as a venue for undergraduate research activities (Barthell et al., 2010; Barthell, 2012a; Barthell et al., 2013; Hensel, 2004; Toufic, 2000).

Researchers have consistently found that interactions with faculty and peers play a major role in determining the quality and impact of a student’s college experience (Pascarella and Terenzini, 2005; Kuh et al., 2006). Such interactions are a natural outgrowth of participation in undergraduate research in the sciences, where students often work in groups in laboratory and field settings. Beyond helping students establish long-term relationships with their mentors, the many benefits of involving undergraduates in research include reinforcing the students’ interest and curiosity in STEM fields, introducing students to the research culture of the academic environment, and helping students apply classroom knowledge across disciplines in real and meaningful settings (Laursen et al., 2010). In turn, actively engaged students are more committed to their studies and the institution, more satisfied with their academic experience overall, and less likely to leave the sciences. Several studies have indicated that students who participate in undergraduate research are significantly more likely (e.g., 80% vs. 59% in the 2003 Bauer and Bennett study) to attend graduate school than those who do not (Summers and Hrabowski, 2006). Moreover, a nationwide assessment of undergraduate research experiences in STEM fields found that the likelihood of a student attaining a STEM graduate degree increases with the duration of the research experience (Russell et al., 2007): only 8% of those surveyed without research experience pursued STEM doctoral degrees, compared to 13% with 1-3 months research

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experience, and 30% having more than one year. Consistent with these findings, the CMS has promoted early and prolonged research experiences for undergraduates. This journal is designed to promote this activity even further, and for the benefit of our students.

Through its Center for Undergraduate Research and Education in Science, Technology, Engineering, and Mathematics (CURE-STEM), which sponsors this journal, the CMS supports faculty development coupled with student learning outcomes through undergraduate research and related curricular changes that help to produce products of scholarship (publications, presentations, etc.). Initiated in 2008 as per the notion of Elgren (2004) and designed to be synergistic with existing programs (e.g., RCSA) that conform to UCO’s goal of providing Transformative Learning opportunities for students, this Center provides resources to faculty (e.g., reassignment time, supplies, and travel funds) who are engaged in student-centered research and their students (stipends and travel funds) with the understanding that they will collaborate to pursue original questions with professional outcomes. In turn, faculty members have garnered $6,669,464 in external grant funding over a six-year period in support of students, successfully pursuing, for example, National Science Foundation REU, STEP, and CURE-S-STEM grants, as well as the CURE-SSS-STEM grant from the Department of Education. A faculty driven and student-centered approach to research is clearly evident from these CURE-STEM Scholars and many other faculty in CMS.

As recently published in the *Council on Undergraduate Research Quarterly*, there has been a demonstrable increase in retention and graduation rates of students participating in the NSF STEP and S-STEM programs, particularly for those engaged in research (Barthell et al., 2013). The first-to-second year retention rate of all students in the 2010 UCO STEP cohort for example was 81%, compared to 96% for the cohort students who were involved in research during their freshmen year and in stark contrast to the overall UCO STEM retention rate of 48%. This increased retention rate also correlates with increased GPAs. The average GPA at the end of the freshman year of STEP participants is higher for those who participated in research: 2.42 for the students not engaged in research during their freshman year, 2.98 for those engaged in research for at most one semester, and 3.15 for those engaged in research for both semesters. Likewise, the average first-to-second year retention rate of the 77 students funded by the current S-STEM grant was 83 percent. The availability of 46 CURE-STEM faculty mentors ensures that every CURE-S-STEM participant has the opportunity to continue their research following the STEP Summer Bridge. While not a program requirement, the majority of Scholars in the current program have elected to do so with compelling results: 87% (34 of 39) participants from the first two CURE-S-STEM cohorts who were involved in research have been retained in STEM majors, compared to only 50% (7 of 14) of those who elected not to participate. Overall, only 15% (9 of 59) of the CURE-S-STEM Scholars who elected to participate in research during (at least) their freshman year have subsequently left the university as compared to 33% (6 of 18) who chose not to participate. Moreover, 47% (7 of 15) of the Scholars who have graduated are currently pursuing advanced degrees.

In the face of fiscally challenging times, a precipitous decline in state funding for higher education and the concomitant increase in tuition and student fees that has necessarily resulted, the CMS has made thoughtful, responsible, and data driven decisions in order to stretch limited resources as far as possible. The return on investment (ROI) over the first three years of the CURE-STEM program was 12.1 to 1, yielding $4,344,538 in external grant revenue for the College. Through a commitment to external fundraising, which has garnered $6,669,464 in external grant funding over a six-year period, as well as $4,633,587 in scholarships and donations, we have ensured that the CMS brought in $1.22 in external funds for every dollar collected from students through course fees over this same time period. These funds have been used to remodel laboratories and classrooms across the College and create interdisciplinary research space for faculty and students, as well as provide support for undergraduates to not only conduct research but to present their work in regional, national, and even international venues. Indeed, members of the CURE-S-STEM Scholar program alone have produced over the past four years 95 student and faculty co-authored presentations at regional and national conferences, 14 conference proceedings papers, and 4 peer-reviewed publications (in *Integrative Cancer Therapies, the Journal of Biomedical Optics, Southwestern Naturalist, and Cell Biology International*).
The number of CMS undergraduate majors has grown by 65.63% over the past seven years and the number of incoming first-time UCO freshmen declaring STEM majors has increased by 9% over the past five years (UCO, 2014). Having strongly emphasized recruiting and retaining members of underrepresented groups through its NSF STEP and S-STEM programs, the CMS has experienced a disproportionate growth over the past few years in numbers of minority students as compared to the rest of the campus and the state. The number of Native American UCO STEM majors has increased by 52.6% (from 97 to 148), while the comparable total for UCO overall has decreased by 8.5% (from 859 to 786); likewise, Hispanic students comprise 7.4% of UCO STEM majors, the number having increased over the last five years by 69.3% (from 153 to 259), while only 5.1% of students enrolled at public institutions statewide are Hispanic (OSRHE, 2014). Likewise, one-fifth of all poster contributions annually to Oklahoma Research Day, regarded as a premier academic research event for undergraduates in the state, are authored by CMS faculty and students. Moreover, 55 undergraduates (44% of those awarded campus-wide) are supported by RCSA grants this fall.

The CMS has further encouraged interdisciplinary discussions and collaboration by initiating over the past few years a CMS Seminar Series, a Research Roundtable, a Center for Interdisciplinary Biomedical Education and Research (CIBER), and a Center for Research and Education in Interdisciplinary Computation (CREIC). These efforts reflect synergies that student-centered research has helped to induce.

The manuscripts that are published in this inaugural journal are an outgrowth of these collaborative efforts among faculty and students. We appreciate the efforts of our colleagues at institutions across the state who have contributed their time and energy to review these manuscripts. However, we are most appreciative of the faculty of CMS, without whom the many advancements in student-centered research seen in the classroom, field and laboratory settings associated with the College would never have been possible.

Enjoy the works contained in this volume. They represent the fruition of a highly collaborative effort in mentorship that deserves the recognition this journal is meant to provide it with.

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Capturing and Analyzing Wheelchair Maneuvering Patterns with Mobile Cloud Computing

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ABSTRACT
Independent mobility is an important factor affecting the health status, quality of life, and social participation of people with disabilities. Power wheelchairs have been widely used to provide independent mobility to such individuals. Despite great advancements in power wheelchairs, wheelchair related accidents occur frequently. Day-to-day wheelchair maneuvering often exposes wheelchair users to confined spaces or other adverse conditions, such as uneven or slippery surfaces, in which even able-bodied people may find it challenging to control a wheelchair. To ensure safe maneuverability, intelligent robotic assistance for wheelchair maneuvering is mandatory. The first step in this process is to capture the wheelchair maneuvering patterns to enable intelligent robotic assistance. In this study, we propose recording, storing, and analyzing wheelchair maneuvering data utilizing mobile cloud computing. Specifically, the accelerometer and gyroscope in smart phones are used to record wheelchair maneuvering data in real-time. Next, the recorded data are periodically sent to the cloud for storage and analysis. The analyzed results are then made available to various types of users, such as mobile phone users, regular desktop application users, etc. The combination of mobile computing and cloud computing leverages the advantages of both techniques and extends the smart phone’s capabilities of computing and storing data via the Internet. To the best of our knowledge, we are the first to use the mobile cloud to capture and analyze wheelchair maneuvering patterns.

I. INTRODUCTION

Independent mobility is important to people of all ages. For example, mobility is an essential indicator for adults with disabilities in evaluating their quality of life and level of participation in social activities. For young children, independent mobility, such as rolling, crawling, or walking, is associated with their social, cognitive, perceptual, and motor development (Ragonesi et al., 2010). Young children with severe motor impairments are at risk for secondary impairments in the aforementioned areas due to locomotor limitations (Kermoian, 1997). To acquire independent mobility, power wheelchairs are widely used. It is estimated that more than 200,000 people in the U.S. use power wheelchairs as their primary means of mobility (Cooper, Summer 2008).

However, wheelchair maneuvering typically requires hand-eye coordination and fine motion control, which is very challenging for people with disabilities, especially for aged and young wheelchair users. Accidents related to wheelchair maneuvering occur frequently and may be accompanied by serious results (McClure et al., 2009; Wang et al., 2009). To provide safe and improved wheelchair maneuvering, intelligent robotic assistance for wheelchair maneuvering is necessary (Demeester et al., 2008). Intelligent robotic assistance consists of various assistance algorithms to intelligently assist different maneuvers (e.g., driving straight forward or backward). Usually, the wheelchair users’ maneuvering intents need to be identified as well in order to choose the most appropriate maneuvering assistance algorithm. Foremost, wheelchair maneuvering data needs to be captured to extract maneuvering assistance patterns and enable accurate and safe intelligent robotic assistance.

This study aims to capture wheelchair maneuvering data, which are used to (1) quantify wheelchair maneuvering patterns for use by assistance algorithms; (2) recover wheelchair movement trajectories to quantify wheelchair users’ daily activities; and (3) gauge activity and participation levels since mobility is essential to ensure regular social activities and improve the quality of life of people with disabilities.

Traditionally, professional accelerometers, e.g., ActiGraph monitors (ActiGraph, 2011), are regularly used to quantify wheelchair maneuvering activities. The accelerometers are installed on the wheelchairs to continuously record maneuvering data for a certain period of time (e.g., one week). The disadvantages of such a data collection scheme are threefold. First, only offline analysis is possible because analysis can only be performed after the data collection pro-
cess is finished. Second, it is hard to manage massive data files. With a sampling duration of one week and a frequency of 30 Hz, 18 million data points would be generated. This amount of data can be difficult to export into portable and manageable files. For example, the management software of ActiGraph supports the export of recorded data into an Excel file. However, older versions of Excel imposed a 65,536 row limit, and although newer versions (2007 and later) have raised this limit to 1,048,576 rows, one week's data still exceeds this size limitation. Third, such a data collection scheme is expensive. Dedicated personnel are required to travel back and forth between the research lab and research participants' homes to install accelerometers on research participants' wheelchairs and periodically fetch maneuvering data. The cost incurred by dedicated personnel and travel is high. In addition, accelerometer sensors are expensive. For example, an ActiGraph package, including software, USB cables, and two monitors, is more than $1,000.

To overcome the aforementioned issues associated with traditional accelerometers, we propose using mobile cloud computing techniques to capture, store, and analyze wheelchair maneuvering data. Accelerometers are typically equipped in smart phones, such as iPhones and Android phones, and such smart phones have become extremely pervasive in the modern world. Therefore, it is practical and convenient to use smart phones to capture wheelchair maneuvering data. The use of smart phones potentially enables more wheelchair users to participate in the research project and alleviates costly travel expenses. More importantly, the cloud can extend smart phones' computational and storage capabilities through the Internet. The combination of these two techniques yields the so-called “mobile cloud computing,” which leverages the benefits of both techniques.

The remainder of this paper is organized as follows. Section II presents our preliminary study findings using traditional accelerometers. Section III presents a general mobile cloud computing framework for data collection, storage, and analysis. Section IV proposes methods for data smoothing and processing, also known as “classification.” Then, the framework is evaluated with a case study in Section V. In section VI future research directions are identified and in Section VII related work by other researchers is reviewed. Finally, in Section VIII we present our conclusions.

II. PRELIMINARY STUDY

A tri-axis accelerometer measures accelerations in three dimensions, namely, x, y, and z with z being the vertical dimension. If we put the accelerometer to one of the wheels of the wheelchair, the z-axis wheel acceleration resembles a sinusoid with peak amplitude of 1g as shown in Figure 1 (Sonenblum et al, 2008).

![Figure 1. z-axis wheel acceleration.](image1)

To ease the calculation of distance, we take the absolute values of the z-axis wheel accelerations, which are shown in Figure 2.

![Figure 2. Absolute values of z-axis wheel acceleration.](image2)

Note that each wave period crosses the 0.5g threshold four times. Thus, the number of wheel revolutions can be calculated by counting the number of times the curve passes this threshold and then dividing by four. To measure the distance of wheelchair movement, we simply calculate the moving distance by:

$$\frac{n}{4} \times \pi \times d$$

(1)

where \(n\) is the number of times the curve passes the threshold 0.5g and \(d\) is the diameter of the wheel.

We used this approach to measure the moving distance of a wheelchair. The results demonstrate that not only could the moving distance be calculated, but the daily activities of the wheelchair user could also be discerned, i.e., whether or not the wheelchair user was able to maintain his/her regular daily activities. However, only using acceleration data in the z-axis has limitations because it cannot depict a complete view of wheelchair users’ daily activities.
Hence, data in more dimensions should be considered.

III. A GENERAL METHOD MOBILE CLOUD COMPUTING FRAMEWORK

In this section, we present a general mobile cloud computing framework designed to overcome the disadvantages of traditional accelerometers, namely, offline-only analysis, massive data files, and high cost.

A. Objectives

Mobile cloud computing makes it possible to conveniently record wheelchair maneuvering data in real-time at low cost. The major elements in the mobile cloud are depicted in Figure 3, including wheelchairs, smart mobile devices, and information recipients, such as mobile users with smart phones, tablet PCs, laptop PCs, etc., and traditional users with desktop PCs.

Smart mobile devices are physically bound to wheelchairs and serve as the information provider. The cloud is responsible for storing and analyzing the maneuvering data; and the information recipients retrieve the analysis results from the cloud, which are shown in different formats (e.g., a table, 3-D trajectory, etc.) to facilitate understanding.

Figure 3. The mobile cloud computing framework

B. Mobile Computing

The use of smart mobile devices in biomedical research has started to emerge. For example, Georgia Tech Research Institute (GTRI) proposed using iPhones to enable “persons with Parkinson’s disease and certain other neurological conditions to use the ubiquitous devices to collect data on hand and arm tremors and relay the results to medical personnel” (GTRI, 2011).

Figure 4. Illustration of 3-D accelerations

In our study, we utilize the accelerometers equipped in smart phones to record wheelchair maneuvering data. The accelerometers can record accelerations in three dimensions (3-D) as shown in Figure 4. The advantages of smart phones include their ubiquitous nature and easy-to-carry characteristics. However, smart phones have relatively limited computing power and small storage capacity. It is impractical to require smart phones to perform online data analysis and store all the recorded data locally.

Fortunately, cloud computing provides a natural extension to mobile computing, i.e., moving data storage and analysis from smart phones into the cloud. Instead of sending the recorded data to the cloud instantly, our application stores the data temporarily in the memory of the phones. Then, the smart phone app writes data stored in memory to a .csv file for every 10,000 data points collected. The same data is simultaneously transmitted to the cloud via an HTTP POST and a JSON array object. Such a batch processing approach brings two additional benefits: (1) It can avoid overloading smart phones from frequent network connections; and (2) It can consequently save power consumption because WIFI or 3G connections consume significant energy (Crk et al., 2009).

C. Cloud Computing

The cloud virtually provides unlimited storage and processing power. Therefore, the cloud in our study assumes two responsibilities, namely, data storage and data processing. 1) Data storage. Periodically, smartphones upload wheelchair maneuvering data to the cloud. These data are subsequently appended to an existing database table, which corresponds to a particular smart phone client. A one-to-one mapping between the smart phone device and its database table in the cloud should be established before data collection begins.
2) Data processing. Given the frequency $f$ of the accelerometer, we assume that the recorded accelerations $(\alpha_x, \alpha_y, \alpha_z)$ remain constant during the time period of $\Delta t = \frac{1.0}{f}$. Hence, we can calculate the updated velocity of the wheelchair using

$$v_w = v_{0w} + \alpha_w \times \Delta t \quad (2)$$

where $v_{0w}$ is the current velocity in dimension $w$ ($w = x, y, \text{ or } z$), and $\alpha_w$ is the acceleration in dimension $w$.

To calculate the moving distance during the time period $[a, b]$ in a particular dimension, we apply Simpson’s rule as follows:

$$\int_a^b v(t) dt = \frac{1}{3} \Delta t [v_0 + v_n + 4(v_1 + v_3 + \cdots + v_{n-1}) + 2(v_2 + v_4 + \cdots + v_{n-2})] \quad (3)$$

where $v(t)$ is the velocity function, which is typically unknown for wheelchair maneuvering; $a$ and $b$ are boundaries of the time period, i.e., $t \in [a, b]$; and $v_i (i=0, 1, 2, 3, \ldots n)$ is the velocity calculated as discussed in (2).

Based on (3), we can calculate the total distance traversed in a particular dimension during a short time period $\delta_t$ (e.g., $\delta_t = 0.5$ second). Combining the calculated results from all three dimensions can reveal the physical displacement in a 3-D space during $\delta_t$. Therefore, putting all such time points together, we are able to draw a 3-D wheelchair movement trajectory for the time period $[a, b]$.

IV. DATA SMOOTHING AND PROCESSING

A. Data Smoothing

Data collected from the smart phone’s accelerometer are very noisy. Even where it should be clear that acceleration is continuously increasing or decreasing over a given time interval, i.e. the wheelchair was accelerating forward or backward, the data appear jagged on a graph making it difficult for a machine or even a human to discern an increasing or decreasing trend in acceleration. For this reason, we chose a simple smoothing algorithm to make the data more human-and-machine readable. The algorithm used in this study is called a sliding-average smooth, shown in (4). It uses the mean of the $n$ nearest data points as the value of the $k_{th}$ data point. An $n$-value of 5 neighboring data points has yielded a much smoother signal than the original as portrayed by Figure 5 and Figure 6.

$$S_k = \frac{Y_{k-2} + Y_{k-1} + Y_k + Y_{k+1} + Y_{k+2}}{5} \quad (4)$$

where $S_k$ is the resulting smoothed data point and $Y_k$ is the current Y-axis point to be replaced by $S_k$.

The smoothing algorithm may need to be performed multiple times in order to attain a properly smoothed signal. For the data we have collected so far, 3 passes with the 5-point smoothing algorithm in (4) seem to provide a sufficiently smooth signal for us to identify a positive or negative trend in acceleration. After conducting the smoothing, the KNN algorithm is still able to identify wheelchair maneuvering patterns accurately.

The dips on either end of the signal in Figure 6 are caused by the inability to smooth the first and last two points due to the nature of the smoothing algorithm requiring, in our case, 2 points before and after the point being smoothed.

It should be noted that the overall amplitude of each point is significantly reduced with data points in this example being reduced from approximately -0.6 to 0.8.
down to around -0.05 to 0.1. This has no effect on our processing algorithm, as explained in the next section.

**B. Data Processing**

In order to classify data into activities such as left and right turns and forward and reverse movements, we selected the K-Nearest Neighbor (KNN) machine learning algorithm. The KNN algorithm classifies a set of data points by calculating the Euclidian distance between the set of target data and multiple sets of sample data. These sample data sets are pre-classified into categories of forward-left, forward-right, forward-straight, backward-left, backward-right, and backward-straight motions and are used as the algorithm’s training data. The target data are divided into pieces, each having the same size as the training samples in terms of the number of data points. The formula in (5) shows how to calculate the Euclidian distance:

\[ \sqrt{\sum_{l=1}^{P} (S^i_l - T^j_l)^2} \quad (5) \]

where \(S^i_l(i = 1, 2, \ldots, m)\) denotes the \(l\)-th data point in the \(i\)-th sample \(S^i\) with \(m\) being the number of samples; \(T^j_l(j = 1, 2, \ldots, n)\) denotes the \(l\)-th data point in the \(j\)-th target data with \(n\) being the total number of pieces in the entire target data; and \(p\) is the number of data points in the sample data \(S^i\) (or equally in the target data \(T^j\)).

For each piece of target data \(T^j\), its distance to each of the sample data is calculated as shown in formula (5). KNN classifies \(T^j\) based on the majority of the \(K\) closest sample data. Despite its simplicity, our experimental results show that KNN is a very effective approach to accurately classifying maneuvering data.

**V. CASE STUDY**

We performed a case study to evaluate the feasibility of the proposed mobile cloud framework as shown in Figure 7. Specifically, we used a Samsung Galaxy SII (GT-I9100) with Android OS 4.0.3 to collect wheelchair maneuvering data. We used Google App Engine (GAE) as the cloud computing platform. GAE enables software platforms to move from their traditional development environments into the cloud. GAE offers Platform-as-a-Service (PAAS), providing an ideal foundation to build robust and scalable Web applications.

The combination of an Android smart phone and GAE provided strong compatibility because they are both developed by Google and provide APIs to developers in order to make the mobile devices work seamlessly with GAE.

Figure 7 shows the data flow in the sequence of data recording, storage, analysis, and results notification. To record data, we set the accelerometer frequency as “SENSOR DELAY UI” (14 ~ 16 Hz) to prevent an overwhelmingly large amount of data collection. Android OS 4.0.3 includes a high-pass filter in its accelerometer APIs, allowing the noise caused by gravity to be filtered out from the recorded data.

Two types of sensor data were collected, gyroscope and accelerometer data. Accelerometer data were used to identify forward and reverse acceleration while gyroscope data were used to identify left and right turns on the wheelchair.

During data collection we performed each of the 6 types of maneuvers 5 times in order to have adequate sample data for our KNN algorithm to accurately identify the type of maneuver being performed on a set of target data. The maneuvers for which we collected data were forward-left, forward-right, forward-straight, backward-left, backward-right, and backward-straight. The data collection was conducted in the corridor of the University of Central Oklahoma Mathematics and Computer Science (MCS) building. During data collection, we drove an Invacare adult power wheelchair while collecting data with the Samsung Galaxy SII smart phone. Video was taken for the duration of the driving data collection in order to facilitate the identification of pattern data. After the data were collected, the smoothing algorithm in Section IV Part A was used on the noisy acceleration data to assist in the identification of the maneuvering data to be used as training data for our KNN algorithm. Ac-
Acceleration data were used to determine the forward and reverse motion of the wheelchair using the Y-axis data taken from the smart phone’s accelerometer. We used the same methods, i.e., KNN, along with Z-axis gyroscope data, to determine if the wheelchair was turning left, right or moving straight forward.

After the collection of every 10,000 data points, the recorded data were saved as a file on the mobile device and immediately uploaded to the cloud. This was done by sending an HTTP POST request with a JSON array object to the server operating on the GAE platform. The server then parses the JSON object and inserts individual data point information (a timestamp and x, y, and z sensor data) into Google App Engine’s Datastore service.

Once all data collection was completed and the acceleration data were smoothed, we developed a KNN to identify wheelchair maneuvering patterns. By using the acceleration data to match forward and reverse motion and the gyroscope data for matching left and right turns, we got a comprehensive view of wheelchair maneuvering activities.

VI. FUTURE WORK

We plan to use the Google Cloud Messaging (GCM) service to enable asynchronous communications between an Android smart phone and GAE as shown in Figure 7. Once the analysis results are available, GAE notifies GCM, which in turn will notify information recipients. As a result, our Android application is not required to periodically check GAE, thereby substantially reducing the number of requests to GAE. The use of GCM avoids long connections with GAE and thus, can reduce the cost incurred by using GAE. Moreover, since the analysis process can be lengthy and the Android application may have already quit running in the meantime, GCM can invoke our Android application to retrieve the analysis results from GAE. To present the analysis results to various types of information recipients, HTML5 will be used in the study as shown in Figure 7. HTML5 is the latest version of HTML, supporting the latest multimedia, including 3-D animations. More importantly, a Web page rendered in HTML5 will be interpreted in the same way by different client programs. Hence, significant effort is saved by avoiding the need to customize the UI presentation for different types of information recipients.

VIII. CONCLUSION

In this paper, we identified a solution to limited computing power of smart phones and the offline analysis disadvantage of traditional sensors. We then proposed a general mobile cloud framework, consisting of wheelchairs, smart mobile devices, cloud services, and information recipients. The smart mobile devices are attached to the wheelchairs. Wheelchair maneuvering data are continuously recorded and periodically sent to the cloud for storage and analysis. Then, the analysis results are made available to various types of information recipients. To effectively filter the noise, we proposed an approach that serves as a preprocessing step for noise reduction. We also conducted a case study to evaluate the feasibility of the proposed mobile cloud framework by using Android smart phones and Google App Engine (GAE). Although the case study is still ongoing, we are convinced that this approach is feasible for conveniently collecting data and performing real-time analysis in the cloud.

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Design of an Inexpensive Particle Image Velocimetry System for Fluid Dynamics Research

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ABSTRACT
Particle image velocimetry (PIV) is a method of studying fluid flow by using particles seeded in a fluid and used as a reference in tracking the fluid behavior. By making a video recording of the flow of a seeded fluid and using software to analyze the video, the fluid behavior can be determined at points within about 0.3mm. This method has become widely used in the past few decades, but purchasing such a system can still cost tens of thousands of dollars. Designing a system with readily available components has drastically reduced this cost. In this paper, the design implementation and accuracy of an inexpensive PIV system developed at the University of Central Oklahoma (UCO) will be discussed.

I. INTRODUCTION
The field of experimental fluid dynamics is a challenging area of study due to the difficulty of measuring the flow of a fluid throughout an entire test section. The test section is a specifically designed channel for studying fluid behavior. Teams at UCO have been studying energy losses in various test sections for the past few years. Until recently, these studies have been completed by measuring only the average flow rate and the pressure losses in the test sections (Henderson et al., 2012). In this paper, we explore these energy losses using a newer method known as entropy generation.

Determining entropy generation in a test section requires that the fluid flow across the entire test section is known (Sanchez et al., 2012). A particle image velocimetry (PIV) system makes the measurement of entropy generation possible by determining the velocity field (Munson et al., 2009) (Schroeder and Willert, 2008). The velocity field is the distribution of velocity vectors as a function of time and position. In order to implement this on a relatively low budget, a PIV system can be designed using cost effective components. For the purpose of our experiments we used a system that closely resembles that built by William G. Ryerson and Kurt Schwenk (Ryerson and Schwenk, 2011).

The four basic components of a PIV system are a light source, seeding particles, a camera, and analysis software. Each of these components can be expensive due to the high quality needed for a PIV system. In our system we used a Casio® Exilim HS Ex-ZR100 that cost $300. This camera can output full high definition (HD) and has a resolution of 1920 x 1280 pixels video at 30 frames per second (fps) and 60, 240, 480, and 1000 fps at reduced resolutions. In our tests we use the full HD setting if the fluid motion is slow, but at higher velocities we typically use the 240 fps setting. The resolution at this setting is 432x280, which is just sufficient for our tests. At higher frame rate settings the resolution is too low for adequate PIV analysis.

II. METHODS
The major parts in PIV systems are the laser, seeding particles, camera, and analysis software. Each of these components can be expensive due to the high quality needed for a PIV system. In our system we used a Casio® Exilim HS Ex-ZR100 that cost $300. This camera can output full high definition (HD) and has a resolution of 1920 x 1280 pixels video at 30 frames per second (fps) and 60, 240, 480, and 1000 fps at reduced resolutions. In our tests we use the full HD setting if the fluid motion is slow, but at higher velocities we typically use the 240 fps setting. The resolution at this setting is 432x280, which is just sufficient for our tests. At higher frame rate settings the resolution is too low for adequate PIV analysis.

The laser used in our experimental setup is a NO-VA Laser © X100 Compact Laser Pointer and X-Series Lens Holder and Optics for $252. This laser is rated a 100mW, which is about 20x more powerful than
typical laser pointers available at department stores. The Lens Holder and Optics allows us to create a laser sheet. Directing the laser perpendicular to the tube allowed minimal diffraction, allowing a truly circular test section to be used. In order to capture the seeding particles better on camera, it may be necessary to use a bandpass filter, but at the present time we have not needed to purchase such a filter. If necessary, a bandpass filter can be purchased for around $75.

One challenging decision in our system was the choice of seeding particles. These particles must be small enough not to affect the fluid flow, and they must also be the correct density and shape to sink/float at a very slow rate – also known as neutral buoyancy. In professionally purchased systems, glass or polystyrene spheres are typically used for water. These spheres are hard to produce and thus costly. Rather than trying to manufacture particles, we simply used a specific type of algae (Spirogyra).

By pulverizing the algae we produced particles from 50-100 microns in size that were essentially neutrally buoyant. Calculations of the terminal velocity of these particles are shown in Table A.1. In this table, the terminal velocity of the algae particles in the water/glycerin mixture is given (Munson, Young et al. 2009); how far the particles drop across the test section for the slowest velocity and a comparison of the fall distance to the hydraulic diameter of the test section are also given in the table. A percentage below five indicates the particles can be considered as neutrally buoyant. At this size and density, their motion in water can accurately be used to depict the actual flow (Stabenau 1976). Spirogyra can be purchased for a modest price; however, we were able to farm and harvest some using an on-campus pond.

The last major component for a PIV system is software. We used an open source program called PIVlab that is run using MATLAB® software for mathematical and technical computing. This software takes a series of images and performs PIV analysis on them (Thielicke and Stamhuis 2012). Figures A.1-3 show two pre-analyzed images and the resulting analyzed image output by PIVlab. In order to extract images from a movie so they can be used in PIVlab, we used an open-source software called Avidemux (http://fixounet.free.fr/avidemux). A list of all components can be seen in Table A.2. A student version of MATLAB® can be purchased for $100 and used with PIVlab.

### Table A.1 Calculations of the terminal velocity of algae particles.

<table>
<thead>
<tr>
<th>Algae Diameter (mm)</th>
<th>Mass (kg)</th>
<th>Channel Length (mm)</th>
<th>Minimum Velocity (mm/s)</th>
<th>Terminal Velocity (mm/s)</th>
<th>Fall Distance (mm)</th>
<th>Percent of Hydraulic Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>6.126E-10</td>
<td>19</td>
<td>2.75</td>
<td>0.008</td>
<td>0.054</td>
<td>0.87</td>
</tr>
<tr>
<td>0.1</td>
<td>4.900E-09</td>
<td>19</td>
<td>2.75</td>
<td>0.002</td>
<td>0.222</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Figures A.1 and A.2** Two pre-analyzed sequential images taken from a PIV video.

**Figure A.3** PIVlab output using figures A.1 and A.2.
Table A.2 Cost associated with each component of the PIV system

<table>
<thead>
<tr>
<th>Component</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casio® Exilim HS Ex-ZR100</td>
<td>$299</td>
</tr>
<tr>
<td>NOVAlaser X100 Compact Laser Pointer</td>
<td>$229</td>
</tr>
<tr>
<td>NOVAlaser X-Series Holder and OpticsKit</td>
<td>$23</td>
</tr>
<tr>
<td>MATLAB® Student version</td>
<td>$100</td>
</tr>
<tr>
<td>PIVlab®</td>
<td>$0</td>
</tr>
<tr>
<td>Avidemux</td>
<td>$0</td>
</tr>
<tr>
<td>Spirogyra</td>
<td>$0</td>
</tr>
<tr>
<td>Dolica 620B100 Tripod</td>
<td>$38</td>
</tr>
<tr>
<td>AAA Batteries</td>
<td>$10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$699</strong></td>
</tr>
</tbody>
</table>

Once all the items were purchased and the system was configured, we needed to test and validate our system. To do this, we set up a test section for fully developed laminar flow in a circular test section. Fully developed laminar flow is a flow characteristic where the fluid flows in layers parallel to each other and not changing with respect to location in a duct or pipe. This type of flow is shown in Figure A.4. Under these conditions, we can accurately predict the average velocity using the pressure drop over a certain distance using the equation

$$ v_{ave} = \frac{R^2 \Delta P}{8 \mu L} \quad (1) $$

where $R$ [m] is the radius of the tube, $\Delta P$ [Pa] is the pressure drop, $\mu$ [(N∙s)/m²] is the dynamic viscosity, and $L$ is the distance the pressure is measured (Munson et al., 2009). We used a water and glycerin mixture to achieve a high dynamic viscosity. The dynamic viscosity of this mixture was $\mu = 0.0118 \pm 0.00002$ (N∙s)/m². The viscosity was measured using a Cannon Instrument Company® viscometer. The test section length was $L = 930 \pm 0.5$ mm and radius $R = 6.35$ mm. The flow profile as a function of radial position can be constructed using the Hagen-Poiseuille flow profile (Bejan, 1984).

$$ V(r) = 2v_{ave}\left(1 - \left(\frac{r}{R}\right)^2\right) \quad (2) $$

We were able to make the pressure measurements using a data acquisition system, shown in Figure A.5, designed for related experiments. The pressure measurements were made up and downstream using differential pressure sensors measuring the difference between the atmospheric pressure and the fluid spaced at $930 \pm 0.5$ mm apart. A schematic diagram of the PIV system can be seen in Figure A.6. PIV analysis was then performed on the flow to determine the experimental flow profile. The Hagen-Poiseuille flow profile was then compared to the PIV determined flow rate by calculating the percent difference at each velocity location. In order to give a more concise result, the total percent difference for each flow profile was determined by averaging the differences obtained at each location.

Figure A.4 Fully-developed laminar flow in a circular test section.

Figure A.5 Printed circuit board used in measuring the pressure drop.
III. RESULTS AND DISCUSSION

The results of comparing the theoretical and experimental fluid velocities can be seen in Table A.3. This table shows the pressure measured, the Reynolds number (defined as the product of fluid density, mean velocity from Eq. (1), and tube diameter divided by fluid viscosity), and the total percent difference of the Hagen-Poiseuille profile compared with the experimental flow profile. In this experiment, a highly viscous fluid was used in order to give a pressure drop that was large enough to accurately measure. In order to achieve this, we mixed approximately a 1:1 volume ratio of water and glycerin. A control valve, shown in Figure A.6, was adjusted to give an average fluid velocity ranging from 2.75 ± 0.4 mm/s to 16.73 ± 2 mm/s.

Table A.3 Total percent difference of the flow profile at various Reynolds numbers.

<table>
<thead>
<tr>
<th>ΔP (Pa)</th>
<th>Reynolds Number</th>
<th>Percent Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1.63</td>
<td>3.4%</td>
</tr>
<tr>
<td>44</td>
<td>2.99</td>
<td>8.4%</td>
</tr>
<tr>
<td>53</td>
<td>3.60</td>
<td>2.2%</td>
</tr>
<tr>
<td>142</td>
<td>9.65</td>
<td>6.6%</td>
</tr>
<tr>
<td>156</td>
<td>10.61</td>
<td>12.8%</td>
</tr>
</tbody>
</table>

The percent differences are very small with the highest being below 12.8%. The last fluid velocity was close to the maximum of our camera’s capability of tracking fluid motion. The significance of these results is that we were able to design a PIV system that is accurate to within 12.8% for average velocity over a specified range. In most test sections we plan to use a hydraulic diameter of 6.35mm, the same as that used in this experiment. Due to similarities we know that the average velocities found in this experiment should correspond to error measurements in our other test sections.

This system is easily upgradable since no piece is confined to the system. Purchasing a camera capable of higher frame rates at higher resolutions would potentially make the system usable at much higher velocities. One issue with this is that slightly better cameras cost significantly more. However, if we acquire funding to do so it will be easy to switch cameras. Our modular system allows a more powerful laser or new optics configurations. This is also expensive, but it is in no way hampered by the system configuration.

IV. SUMMARY AND CONCLUSION

Cost effective components of a PIV system were found. These were then assembled into an entire cost effective PIV system that has a component cost that can be seen in Table A.2. The entire system was then tested against a known model of fully developed laminar flow in a circular tube. The results are given in Table A.3. The results show an error less than 12.8%, indicating an accurate system over the specified range of Reynolds numbers.

We found that by using cost effective materials it is very possible to build a PIV system that rivals professionally designed systems. This is important because it greatly increases the accessibility of a PIV system allowing a wider range of entities to perform experimental fluid dynamics research. For our research team, looking into entropy generation in junctions is now possible, a long term goal that will hopefully soon be realized.

ACKNOWLEDGMENTS

This research was made possible in part by the support of the UCO Student Research, Creative, & Scholarly Activities (RCSA) grant program and the National Science Foundation (NSF) STEM Talent Expansion Program (STEP) on the UCO campus. Aric Gillispie and other incoming freshmen assisted in this work during the STEP program. A special thanks to Dr. James Bidlack (UCO Biology) for extending his expertise in growing algae. The faculty advisor for this paper was Dr. Evan Lemley (UCO Engineering and Physics), without whose knowledge and expertise in experimental fluid dynamics none of this research would have been possible.
REFERENCES


BIOGRAPHIES

Brock Ring

Brock P. Ring is a class of 2014 undergraduate Engineering Physics – Mechanical Systems student. He has worked in Dr. Evan Lemley’s fluid dynamics laboratory since the fall of 2012. Upon graduation, Mr. Ring plans on joining the workforce on track to become a professional engineer.

Andrew Henderson

Andrew W. Henderson graduated in 2013 with a B.S. degree in Engineering Physics from the University of Central Oklahoma. He worked extensively in the fluid dynamics laboratory of Dr. Evan Lemley throughout the majority of his undergraduate studies. After graduation, Mr. Henderson accepted a manufacturing engineer position at the Goodyear Tire and Rubber manufacturing plant in Lawton, Oklahoma.

Daniel Atkinson

Daniel K. Atkinson is an undergraduate Engineering Physics – Electrical Systems class of 2015 student. He began working in Dr. Evan Lemley’s fluid dynamics laboratory his freshman year and has continued this work throughout his time at the University of Central Oklahoma. Upon graduation, Mr. Atkinson plans on going into industry and working as an electrical engineer.

Evan Lemley

Evan Lemley is a Professor of Engineering and Physics at the University of Central Oklahoma (UCO) and serves as an Assistant Dean in the UCO College of Mathematics and Science. He earned his B.A. in Physics from Hendrix College, and his M.S. in Mechanical Engineering and Ph.D. in Engineering with emphasis in Nuclear and Thermal-Fluid Systems from the University of Arkansas.
ABSTRACT

Jasmonic acid (JA), a plant hormone, has the potential to alter production of bioenergy crops through regulation of enzymes that affect plant growth and metabolism. Exogenous applications of JA (0.5 to 1.5 mM) to sorghum [Sorghum bicolor (L.) Moench] and switchgrass (Panicum virgatum L.) were evaluated to determine its effect on fresh weight (FW) and dry weight (DW) biomass, and activities of hydroxymethylglutaryl CoA reductase (HMGR) and phenylalanine ammonia lyase (PAL). The FW of switchgrass, as well as the DW of both species, decreased significantly, with a 63.3% reduction in DW of switchgrass treated with the 1.5 mM JA concentration. Data from HMGR assays followed a reverse trend; increasing JA concentration increased the activity of HMGR two-fold in sorghum, and moderately in switchgrass. Activity of PAL was not significantly affected by JA treatments in sorghum, but PAL activity was reduced to almost half the value of controls in switchgrass. Exogenous application of JA in sorghum and switchgrass generally decreased biomass and increased HMGR activity, with either no effect or a decrease in PAL activity. Decreased biomass production may be offset by increased resilience to pests in the field and a more favorable energy conversion of the harvested biomass.

Key Words: Jasmonic acid, HMG-CoA reductase, PAL, sorghum, switchgrass

I. INTRODUCTION

Desirable benefits for the production of biofuel from the annual row crop, sorghum [Sorghum bicolor (L.) Moench], and the perennial forage crop, switchgrass (Panicum virgatum L.), have been reviewed recently (Linton et al., 2011; McLaughlin and Kszos, 2005). Sorghum and switchgrass are drought-resistant, warm-season C-4 species that may be cultivated on marginal lands, providing a higher biomass yield compared to conventional food crops (Varvel et al., 2008). While there is potential for these species as biomass crops, there are problems, including cultivation under exceptionally-dry conditions, vulnerability to pests, and processing in the conversion to biofuel.

Jasmonic acid (JA), which is a lesser-known natural plant hormone (Bidlack and Jansky, 2014), has the potential to regulate a myriad of growth processes and metabolic pathways. Jasmonic acid (JA) and its derivatives (collectively called jasmonates) are stress-induced hormones that modulate the expression of genes for the production of secondary compounds (Creelman and Mullet, 1997). Two such enzymes are hydroxymethylglutaryl CoA reductase (HMGR) of the mevalonic acid pathway and phenylalanine ammonia lyase (PAL) of the shikimic acid pathway. The HMGR and PAL enzymes regulate synthesis of terpenoids involved with pest resistance and phenylpropanoids involved with cell wall synthesis, respectively (Bidlack et al., 1994; Yoshioka and Doke, 1994). As such, jasmonates that regulate these two enzymes are inherently responsible for the ability of plants to deter herbivory and make-up of the cell wall that ultimately affects conversion to biofuel. There may, indeed, be potential for commercial use of JA, if it enhances pest resistance and improves biomass crop conversion to biofuel.

The effect of JA has not been studied extensively in C-4 plants and the role of JA in altering HMGR and/or PAL in sorghum and switchgrass has not been reported in the literature. The objective of this experiment was to determine if application of exogenous JA significantly affects biomass accumulation, as well as HMGR and PAL activities, and if so, whether the effects are negative or positive.

II. MATERIALS AND METHODS

Seeds were germinated and plants were maintained in 30.5-cm diameter clay pots containing a 50:50 mixture of Fafard Soil Mix No. 2 (Conrad Fa- far, Inc., Agawam, MA) and Dale silt loam (fine-silty, mixed, thermic Pachic Haplustoll) with a pH of 6.6. ‘Hegari’ sorghum [Sorghum bicolor (L.) Moench], obtained from Ross Seed Company (El Reno, OK),
and ‘Alamo’ switchgrass (*Panicum virgatum* L.), obtained from the USDA-ARS Grazinglands Research Laboratory (El Reno, OK), were planted in the clay pots in early June 2010. Two weeks after emergence, sorghum plants were thinned to 10 seedlings and switchgrass plants were thinned to 100 seedlings per pot. Granular N-P-K fertilizer was applied to the surface of the soil mixture after the fourth node appeared in the sorghum and the third node appeared in the switchgrass, resulting in the application of 150 kg N/hectare, 60 kg P₂O₅/hectare, and 30 kg K₂O/hectare. Pots were arranged in a randomized complete block design with three replications of the two species, each of which included four jasmonic acid treatments, for a total of 24 pots for the experiment.

All reagents for experimental treatments and plant analyses were obtained from Sigma-Aldrich (St. Louis, MO). The plants were sprayed with jasmonic acid (JA) at four weeks after emergence. Exactly 50 mg of JA was dissolved in 5.0 mL of methanol and then diluted with deionized water to prepare solutions containing 0.5, 1.5, and 5.0 mM JA. Each JA treatment contained 0.10% Triton-X surfactant to promote absorption of the chemical. A control treatment without JA, but containing equivalent concentrations of methanol and surfactant, was also included. Individual pots were moved to an isolated area and the leaves and stems sprayed with 10.0 mL of their designated treatment. The resulting treatments provided for 0.0, 0.005, 0.0015, and 0.050 mmol of JA applied per pot.

In late July/early August 2010 (approximately 60 days after emergence), plants were cut at pot level and weighed immediately to determine total above-ground fresh weight (FW). After removing 5.0 g of fresh material for enzyme analyses, remaining harvested material was dried at 45°C for 5 days and then weighed to obtain dry weight (DW). Percent moisture was calculated as (FW–DW)/FW.

Exactly 5.0 g of plant material from the basal 10 cm of growth was removed from each pot and put on ice for enzyme extraction. The fresh samples of basal tissue were homogenized within 1 hour in 30 mL of a 50 mM Tris buffer (pH = 7.0) containing 0.1 M sucrose, 1% polyvinylpyrrolidone, 4.0 mM cysteine, and 1 mM DTT (modified after Elmer and Bidlack, 2010). The resulting homogenate was strained through four layers of cheese cloth and centrifuged at 5,000 g for 10 minutes, to isolate a crude microsomal fraction (in the supernatant), containing HMGR. The pellet was discarded and the remaining supernatant was centrifuged at 10,000 g for 10 minutes to produce a new supernatant that included cytosolic PAL; the final pellet was discarded.

Activity of the HMGR enzyme was assayed spectrophotometrically. Hydroxymethylglutaryl CoA reductase oxidizes NADPH and reduces 3-hydroxy-3-methylglutaryl CoA to produce NADP+ and mevalonic acid. The HMGR enzyme assay was performed at 30 °C by mixing microsomal isolations with 3-hydroxy-3-methylglutaryl CoA and observing the decrease in absorbance of NADPH at 340 nm, using an extinction coefficient of 6.22 mM⁻¹ cm⁻¹ (two NADPH molecules are consumed in the reaction). The resulting enzyme activities were reported in micromoles of mevalonic acid produced per gram of fresh tissue per hour.

Activity of the PAL enzyme was measured spectrophotometrically. Phenylalanine ammonia lyase catalyzes the production of trans-cinnamic acid from phenylalanine. The PAL enzyme assay was performed at 30 °C by mixing cytosolic isolations with phenylalanine and observing the increase in absorbance of trans-cinnamic acid at 290 nm, using an extinction coefficient of 9.63 mM⁻¹ cm⁻¹. The resulting enzyme activities were reported in micromoles of trans-cinnamic acid produced per gram of fresh tissue per hour.

Data including FW, DW, and percent moisture, as well as HMGR and PAL activities, were analyzed by SAS PROC GLM (SAS Institute, 1985) as a split-plot, randomized complete block design with species as the main effect and exogenous JA application as the secondary treatment tested against residual error. Least significant difference (LSD) tests were calculated to determine significant differences among JA treatments within each species.

### III. RESULTS

Analysis of variance revealed significant differences between species for FW, DW, percent moisture, and HMGR activity (Table 1). Significant differences were also detected among JA treatments for FW as well HMGR and PAL activities. Only HMGR activities varied significantly for the species X treatment interaction.

Within species, significant differences were detected for FW and DW (Table 2), as well as HMGR and PAL (Table 3) activities. No differences in percent moisture were detected within either species, although averaged across JA treatments, percentage moisture of switchgrass (80%) was greater than sorghum (71%).

Both sorghum and switchgrass FW decreased as a re-
result of JA treatment, with a significant decrease in FW of the 5 mM JA treatment compared to the control (Table 2). The decrease in sorghum FW from 1,516 grams per pot in control treatments to 1,322 grams per pot in the 5.0 mM JA treatment was a 12.8% reduction in FW biomass yield; whereas the decrease in switchgrass FW from 391.7 grams per pot in control treatments to 168.3 grams per pot in the 5.0 mM JA treatment was a 57.0% reduction in FW biomass yield. The more dramatic decrease in FW of switchgrass compared to sorghum was also observed in DW results, in which switchgrass DW was 63.3% less in the 5 mM JA treatment (compared to the control), but sorghum DW was not significantly affected by any level of JA treatment.

Activities of HMGR were generally greater in switchgrass compared with sorghum, whereas, activities of PAL spanned a wider range in switchgrass than they did in sorghum (Table 3). The HMGR activities in both sorghum and switchgrass followed the opposite trend observed with biomass results, in that, HMGR activity generally increased with increasing JA concentration, but biomass generally decreased as JA concentration increased. In contrast, PAL activity in sorghum was not significantly affected and decreased in switchgrass when JA was applied. The range of values for HMGR activity in sorghum (6.62 to 15.1 micromoles per gram FW per hour) was greater than those of switchgrass (16.4 to 21.8 micromoles per gram FW per hour); whereas the range of values for PAL activity in switchgrass (2.77 to 5.21 micromoles per gram FW per hour) was greater than the range of those for sorghum (4.24 to 4.77 micromoles per gram FW per hour). These results demonstrated that JA treatment significantly increased HMGR activities in both species (especially sorghum), but significantly decreased PAL activities in only switchgrass.

**Table 1.** Significance of effect in analysis of variance of fresh weight (FW), dry weight (DW), percent moisture (MOIST), hydroxymethylglutaryl CoA reductase (HMGR) activity, and phenylalanine ammonia lyase (PAL) activity as influenced by species and jasmonic acid treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>FW</th>
<th>DW</th>
<th>MOIST</th>
<th>HMGR</th>
<th>PAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (S)</td>
<td>1</td>
<td>**</td>
<td>**</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Rep</td>
<td>2</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Error A</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (T)</td>
<td>3</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>S x T</td>
<td>3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>Error B</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, **Significant at the 0.05 and 01 probability levels, respectively. NS = not significant.

**Table 2.** Fresh weight (FW), dry weight (DW), and percent moisture (MOIST), as affected by jasmonic acid (JA) treatment, on a per pot basis, of sorghum and switchgrass plants, after 60 days of growth.

<table>
<thead>
<tr>
<th>JA concentration</th>
<th>FW (g)</th>
<th>DW (g)</th>
<th>MOIST</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 mM</td>
<td>1516 a</td>
<td>445.0 a</td>
<td>73.19 a</td>
</tr>
<tr>
<td>0.5 mM</td>
<td>1508 a</td>
<td>416.7 a</td>
<td>72.51 a</td>
</tr>
<tr>
<td>1.5 mM</td>
<td>1472 a</td>
<td>415.0 a</td>
<td>69.67 a</td>
</tr>
<tr>
<td>5.0 mM</td>
<td>1322 b</td>
<td>400.0 a</td>
<td>68.89 a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>JA concentration</th>
<th>FW (g)</th>
<th>DW (g)</th>
<th>MOIST</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 mM</td>
<td>391.7 a</td>
<td>90.0 a</td>
<td>81.97 a</td>
</tr>
<tr>
<td>0.5 mM</td>
<td>355.0 a</td>
<td>70.0 ab</td>
<td>79.87 a</td>
</tr>
<tr>
<td>1.5 mM</td>
<td>270.0 ab</td>
<td>56.67 ab</td>
<td>79.27 a</td>
</tr>
<tr>
<td>5.0 mM</td>
<td>168.3 b</td>
<td>33.33 b</td>
<td>77.56 a</td>
</tr>
</tbody>
</table>

†Means for each species within a column with the same letter are not significantly different.

**Table 3.** Hydroxymethylglutaryl CoA reductase (HMGR) and phenylalanine ammonia lyase (PAL) activities in units of micromoles per gram of fresh weight per hour, as affected by jasmonic acid (JA) treatment, of sorghum and switchgrass plants after 60 days of growth.

<table>
<thead>
<tr>
<th>JA concentration</th>
<th>HMGR</th>
<th>PAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 mM</td>
<td>6.617 c</td>
<td>4.773 a</td>
</tr>
<tr>
<td>0.5 mM</td>
<td>11.38 b</td>
<td>4.240 a</td>
</tr>
<tr>
<td>1.5 mM</td>
<td>11.39 b</td>
<td>4.510 a</td>
</tr>
<tr>
<td>5.0 mM</td>
<td>15.12 a</td>
<td>4.663 a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>JA concentration</th>
<th>HMGR</th>
<th>PAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 mM</td>
<td>18.52 b</td>
<td>5.213 a</td>
</tr>
<tr>
<td>0.5 mM</td>
<td>16.40 c</td>
<td>3.237 b</td>
</tr>
<tr>
<td>1.5 mM</td>
<td>18.10 bc</td>
<td>3.877 ab</td>
</tr>
<tr>
<td>5.0 mM</td>
<td>21.70 a</td>
<td>2.767 b</td>
</tr>
</tbody>
</table>

†Means for each species within a column with the same letter are not significantly different.

**IV. DISCUSSION**

Differences observed in this investigation highlight the benefits and drawbacks of employing warm-season annual crops (sorghum) compared to hardy perennials (switchgrass). Unequal biomass productivities were expected between species because of variations in annual and perennial growth habits. Sorghum, an annual, relies on extensive biomass accumulation for competitive survival and reproduction within a single growing season, while switchgrass, a perennial, is...
unlikelihood to devote energy to extensive above-ground biomass accumulation during the establishment. The long term biomass yield of switchgrass over several years without the necessity of repeated annual seeding and establishment must be weighed against the immediate boon of fast-growing crops, such as sorghum, that require intensive annual care and resources. Observation of differences in annual biomass yield and annual labor demands should guide appropriate crop choice with regard to local resources and climate.

Biomass was significantly reduced by JA application in this experiment, although the two species demonstrated differential responses to the JA treatment concentrations applied. Jasmonic acid treatment failed to elicit significant DW responses in sorghum, although there was a decrease in FW at the greatest level of 5 mM JA. In contrast, JA treatment elicited substantial biomass responses in switchgrass with significant decreases in both FW and DW being observed in response to the 1.5 and 5.0 mM concentrations. Species-specific sensitivities to JA applications could be due to inherent differential receptiveness to method of JA application or JA regulation, and developmental growth stage at the time of treatment. Regardless of the differences between species, biomass was generally reduced when JA was applied to the container-grown plants. Further research is necessary to determine if this apparently negative biomass response also occurs when these species are field grown. The mechanism through which JA affected plant growth and its potential application for altering enzyme activity, may promote its use in special situations.

Previous investigations have demonstrated that JA retards biomass accumulation in Canada goldenrod (Solidago canadensis L.) (van Kleunen et al., 2004), whereas other studies have shown that JA has no significant effect on plant weight of tomato (Lycopersicon esculentum Mill.) when pests are absent (Iverson et al., 2001). Both studies, however, provide evidence that JA promotes pest resistance and, in the presence of pests, the biomass of tomato plants treated with JA is greater than those not treated with JA (Iverson et al., 2001). As such, it is possible that crops grown for bioenergy may react differently, particularly in cases when pests are present, because of changes in enzyme activities that ultimately deter herbivory.

The JA treatments in our study increased HMGR activity in both species indicating that in these species, factors affecting HMGR activity are responsive to modulation by JA. Moreover, JA treatments increased HMGR activity in sorghum, and to some extent in switchgrass, in a concentration dependent manner. This positive relationship indicates that a wide range of JA treatments may be used to increase HMGR activity with reliability in sorghum and high concentrations of JA have the potential to increase HMGR activity in switchgrass.

Differences in PAL activity as a result of JA treatments were detected in switchgrass but not in sorghum, suggesting species-specific metabolic responses to JA. This could be due, in part, to the nature of the tissues sampled, particularly because switchgrass contained more leafy material and sorghum included mostly stems. Interestingly, sorghum HMGR activity still varied significantly in this stem material. In switchgrass, both HMGR and PAL were significantly affected by JA treatment and there was a greater range of PAL activity in this species than that of HMGR. As such, these results indicate that sorghum HMGR is sensitive to JA treatment, whereas switchgrass HMGR, and especially PAL, are both sensitive to JA treatment.

Because HMGR is generally responsible for terpenoid production and PAL is generally responsible for phenolic production, results from this study have several implications. This is particularly exciting since JA and its derivatives modulate genes for the production of these types of compounds. From an applied standpoint in biofuel production, it may be advantageous to increase HMGR activity in order to promote pest resistance and hence the yield of bioenergy crops. It might also be advantageous to decrease PAL activity, which has the potential to weaken the cell wall and thus make it easier to use plant material for bioenergy conversion. As such, JA has the potential to regulate pest resistance and plant growth dynamics, both important aspect of plants considered for bioenergy. Although JA decreased biomass in both species for this experiment, JA increased HMGR in both species and even decreased PAL in one species. Even though JA may decrease biomass, this negative effect could be offset by an enhanced resilience to pests and more favorable cell wall composition needed for conversion of biomass to energy.

V. CONCLUSION

Jasmonic acid treatment may increase cellulose quality of biomass crops, but the concentration should be moderated to avoid excessive losses in biomass yield. The effects of various concentrations of JA acid on sorghum and switchgrass indicated that JA is an effective
regulator of HMGR in both species. Therefore, JA may potentially be used to enhance pest resistance in these species. In addition, the results from PAL activity suggest that some concentrations of JA may be an effective regulator of phenolic products, which could lead to improving energy conversion of biomass in switchgrass.

ACKNOWLEDGEMENTS

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BIOGRAPHIES

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Jocelyn Bennett Bidlack received grants from UCO and Oklahoma’s EPSCoR program to provide a modest salary and supplies to support her research as an Undergraduate Biology Major at the University of Central Oklahoma (UCO). After completing dual degrees in Biology and Philosophy from UCO, Jocelyn became a Graduate Student in the Biology Department and is currently working on a bioremediation project for her Advanced Degree.

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Jim Bidlack is a Professor of Biology and CURE-STEM Scholar at the University of Central Oklahoma (UCO). He has been at UCO for over 20 years and has supervised about 100 undergraduate researchers in many areas including agriculture, bioremediation, cell wall chemistry, gene transfer, growth regulation, plant anatomy, plant physiology, and solar energy conversion. He was one of UCO’s first professors to have research projects funded by agencies such as EPSCoR, NSF, OCAST, and USDA. His Research Group continues to thrive with about a dozen undergraduate researchers at UCO.
Fibroblast Contraction is Dose-Dependent on Serum in an Updated Stress-Relaxed Collagen Lattice Model

Jing X. Herwig, Melville B. Vaughan
Department of Biology

ABSTRACT
Fibroblast contraction is an important part of wound healing and pathological contractures. The three-dimensional collagen lattice is widely accepted as the best model to study the cell biology of fibroblast contraction. As a stress-relaxed model, the collagen lattice provides insights into the interaction between fibroblasts and the cytoskeletal complexes with the extracellular matrix. Our study updates the stress-relaxed model from previous reports with new techniques, incorporating digital imaging and software for analysis. Additionally, we demonstrate the serum-dependency of fibroblast contraction to propose a question about the correlation between Fetal Bovine Serum (FBS) and other contraction-promoting agents that may be present in serum, since the exact components and mechanisms of serum stimulation are still unclear. The ease and accuracy of the updated collagen lattice model will provide more robust datasets to study the cell biology of fibroblast contraction.

I. INTRODUCTION
Fibroblasts are the cells that organize connective tissues and synthesize new extracellular matrix (ECM) during wound contraction (Tomasek et al., 2002). When the wound is formed, fibroblasts migrate to the surface of the wound, and start to secrete and organize collagen to form a granulation tissue (Grinnell, 1994). In the early granulation stage, more collagen fibers are recruited, along with other contractile elements like fibronectins to build up physical stimuli in terms of mechanical tension. With the accumulation of tension, fibroblasts may differentiate into specialized contractile cells termed protomyofibroblasts. The protomyofibroblasts express cytoplasmic actins that bundle to form stress fibers to generate more tension (Tomasek et al., 2002). In the later granulation stage, under the presence of chemical stimuli such as transforming growth factor-β (TGF-β), and in contact with the secreted ED-A FN, myofibroblasts are differentiated (Serini et al., 1999; Masur et al., 1996; Desmouliere et al., 1993). The differentiated myofibroblasts are capable of expressing alpha-smooth muscle actins (α-sm actin), similar to the ones produced by smooth muscle cells, and organize the actin microfilamentous apparatus. The expression of α-sm actin is recognized as the major distinguishing characteristic of myofibroblasts. Myofibroblast contraction occurs by the cooperation of major contractile elements, including stress fibers, focal adhesions, and fibronectin fibrils (Hinz et al., 2001a).

Tension generation is considered a cell-mediated event and its study requires appropriate experimental models. Much of what we know about fibroblasts is based on the study of a two-dimensional (2-D) model, which only hints at the fibroblast’s contractile mechanism and activity (Grinnell, 2003). After the discovery that contraction activity occurs at the tissue level instead of cell surface, research demonstrated that the contractile myofibroblasts promote contraction with the help of other various contractile components in the ECM (Beningo et al., 2004) (Hinz, 2010). The 2-D model is clearly not capable of monitoring the interaction between myofibroblast and its environment during contraction (Grinnell, 2003). A new three-dimensional model, the collagen lattice, was developed, having a tissue-like environment that enabled measurement of contraction at the tissue level and monitoring more cell activities: traction, contraction and cell migration (Griffith et al., 2006). The collagen lattice resembles wound-contracting dermis, and can be applied to study wound repair, skin cancer and breast cancer (Bell et al., 1979). These features provide evidence that the collagen lattice model has similarities to in vivo tissues unlike the 2-D model and is therefore a more-appropriate experimental model.

Floating and anchored collagen lattice models represent dermis and contracting wound, respectively (Bell et al., 1983). The floating collagen lattices provide details on the mechanisms of cell traction (which resembles migration) instead of cell contraction (Grinnell, 2000). DNA synthesis (leading to cell
division) differs between the anchored and floating collagen lattices, with a decline of DNA synthesis in the floating collagen compared to that in anchored collagen (Rosenfeld et al., 2000). The tension is generated isotropically in floating collagen while it is generated anisotropically in anchored collagen. The stress-relaxed collagen lattice combines the characteristics of both models, which is divided into the anchored contraction with a reduction in height and the tension-relaxed contraction with a reduction in diameter (Grinnell, 1994), and can be used to study the interplay between resting and wounded dermis.

Here we update the analysis method of the stress-relaxed collagen lattice model, previously reported by Tomasek, to study the stress-relaxed contraction of myofibroblasts (Tomasek et al., 1992) (Vaughan et al., 2000). In the previous analysis method, the contraction of collagen lattice was measured in the form of diameter with a ruler, which increased the possibility of experimental error. The new analysis method mainly employs computer and high-tech microscopy to collect data, which is more objective. The goal of the experiment is to examine the morphological change induced by the contraction in collagen lattice after release. Furthermore, we test the dependency of tension release on serum to discuss the influence of growth factors on stimulating the tension generation and tension-relaxed contraction. The results indicate that serum stimulates fibroblast contraction in a dose-dependent manner.

II. MATERIALS AND METHODS
A. Cell Culture
Human fibroblasts were subcultured from immortalized cultures of Dupuytren’s disease fibroblasts in the lab (DP147 hTERT). Cells were cultured in a 37°C and 5% CO₂ incubator using 100 mm² tissue culture dishes in medium composed of DMEM (Hyclone) +10%FBS (Biowest) + antibiotics (Sigma). Cells were kept in log phase growth and used when 80% confluent. All procedures related to this study were done in compliance with the UCO Institutional Review Board.

B. Preparation of Collagen Lattices
Fibroblasts were cultured within collagen lattices with a final collagen concentration of 0.65mg/ml (rat tail type I collagen; Becton Dickinson) and final cell concentration of 1.25×10⁵ cells/ml as previously described (Tomasek et al., 1992) (Vaughan et al., 2000). The collagen lattices were dispensed in smaller volumes than previously reported (150μL instead of 250μL) in order to accommodate the limitations of the microscope and camera used to photograph the lattices for measurement. Each amount of 150μl of collagen/cell mixture was plated onto a 40mm² tissue culture dish (TPP; Midwest Scientific). After allowing the lattices to gel at 37°C for 1 hour, they were immersed in 2ml culture media with different concentrations of serum. For the regular collagen lattice contraction test, 10% serum was added. For the effect of serum test, 0 and 10% serum were added respectively. For the serum dose-dependent test, 1%, 5% or 10% serum were added over the gelated collagen lattice in different groups respectively. Lattices were incubated at 37°C for 5 days, remaining attached, with the addition of fresh media after 2.5 days.

C. Collagen Lattice Contraction
After 5 days, collagen lattices were photographed then released mechanically from the substratum; first, the edges were lifted with a dissecting needle, then media was gently pipetted under the lifted edge of the lattice to detach the remainder. Lattice area measurements were done from photographs rather than using a ruler to measure diameters, as previously reported (Tomasek et al., 1992) (Vaughan et al., 2000). Culture dishes were kept in the incubator throughout the experiment, except when photographing the lattices.

D. Microscopy and Image Analysis
The rapid contraction of collagen lattice was viewed with an Olympus SZ61 stereo dissecting microscope. The images of lattices were photographed with a digital camera (Insight; Diagnostic Instruments) and SPOT software (Diagnostic Instruments). All the images were processed digitally with the ImageJ computer program (1.46r; NIH). Images were encircled to measure the lattice area in square millimeters after calibrating the software at 87 pixels per millimeter. Outputs were analyzed with scientific data analysis and graphing software (SigmaPlot; Systat Software Inc.). Images were collected prior to release, and at 1, 2, 10, 30, and 60 minutes after release. For whole lattice images, an 8-megapixel digital camera (HTC One X; HTC) captured images through the eyepiece of an inverted light microscope (Micromaster; Olympus).
III. RESULTS
A. Rapid Contraction of Fibroblasts in Released Collagen Lattices

Previous studies demonstrated that fibroblasts will rapidly contract a collagen lattice when tension is released (Mochitate et al., 1991) (Tomasek et al., 1992). Here we observed a similar fast contraction under similar conditions. Replicate lattices demonstrated 42% of the contraction occurred in the first 2 minutes, the time when stress fibers provide the contractile force during stress relaxation (Tomasek et al., 1992) (Figure 1). 10 minutes after lattice release, when most stress fibers have dispersed, we measured a reduction of 50% in surface area (Figure 1). Between 10 and 30 minutes, we observed a slow period with a reduction of only 15% in surface area. At the 30-minute time point, the cells reached the contractile homeostasis stage, which implied that the cell lost most of its stress fibers and maintained the condition until the end of the experiment, as demonstrated in literature. (Tomasek et al., 1992) (Brown et al., 1998). During this time, tractional force continues to reduce the diameter of the lattice, albeit very slowly.

Figure 1. Analysis of collagen lattice contraction under standard conditions. Lattices were incubated attached for 5 days with media plus 10% serum as previously described (Tomasek et al., 1992). The contraction result was comparable to the previously published research. Data points are averages of 9 replicate lattices, +/- standard deviation.

B. Serum Stimulates Rapid Collagen Lattice Contraction

FBS served as an agonist to stimulate the fibroblast contraction in previous studies (Tomasek, 1992). Our experiment tested collagen lattice contraction in the presence or absence of 10% FBS. In the absence of FBS there was little to no contraction, while contraction measurably occurred in the presence of FBS (Figure 2). The lattices treated with no FBS exhibited small number of cells, and most of them were rounded (Figure 3a). In contrast, the lattices with FBS displayed abundant, well-spread cells (Figure 3b), which indicated the generation of tension. These results suggest that FBS stimulates cell growth correlated with tension generation over time.

Figure 2. Analysis of collagen lattice contraction in the presence or absence of serum. For each treatment group, 5 replicate lattices were graphed as averages, plus or minus standard deviation. The concentration of serum added into the lattice after gelation was 0% (●) and 10% (○). The lattices with no serum exhibited little or no contraction. The lattices with 10% serum exhibited a 70% reduction in contraction. The homeostasis occurred after 30 minutes, when the maximum amount of stress relaxation had occurred.

Figure 3. Representative light micrographs of cells in collagen lattices. A: After 5 days, the cells in lattices treated with no
serum were rounded, and displayed a decline in cell number. B: After 5 days, the cells in lattices treated with 10% serum were spreading out, and displayed an observable increase in cell number. 100x total magnification.

C. Dose-Dependent Rapid Contraction
We tested whether there was an FBS dose-dependent response of cell contraction by incubating lattices in the presence of media with 1%, 5%, and 10% FBS. The contraction ability of fibroblasts varied in response to different FBS concentrations (Figure 4, 5). Lattices visibly contracted more in media with 5% and 10% FBS than lattices in media containing 1% FBS (Figure 4). A tenfold decrease in FBS concentration reduced contraction by half (Figure 5). Interestingly, contraction in response to 5% and 10% FBS was similar, either the cells had reached their maximal ability to contract, or to respond to serum. These results demonstrate that stress-relaxed collagen lattice contraction is FBS dose-dependent and appears to have a maximum dose limit.

**Figure 4.** Representative images of collagen lattices demonstrating the morphological change caused by the fibroblast contraction at 0, 1, 2, 10, 30, and 60 minutes after release. Three series of images are the collagen lattices treated with 1%, 5% or 10% FBS for 5 days prior to release. Magnification 6.7x.

**Figure 5.** Analysis of dose-dependent collagen lattice contraction with different serum concentrations. For each treatment group, 6 replicate lattices were graphed as averages, plus or minus standard deviation. The contraction stimulated by serum is a dose-dependent process. The concentration of serum added into the lattice after gelation was 1% (●), 5% (○) or 10% (▼). The rapid contraction occurred in the first 10 minutes. The slow traction occurred between 10 to 30 minutes. The homeostasis occurred after 30 minutes.

IV. DISCUSSION
The stress-relaxed model described by Grinnell (1994) combines the anchored lattice and relaxed lattice into a process to provide the insights of fibroblast contraction under stress. The combination of both models also demonstrates the transition from granulation tissue to dermis, which benefits wound healing study (Grinnell, 1994, 2003, 2010). With this model, the mechanical contraction process can be observed and measured in a scientific method to understand cell-mediated fibroblast contraction (Tomasek 1992). Furthermore, this model is used for the study of interactions between a cell and its ECM, the mechanisms of traction and contraction, and the function of contractile elements (Tomasek 2002).

Our primary goal here was to update methods previously used to study stress relaxation (Tomasek et al., 1992) (Vaughan et al., 2000). In the previous studies, collagen lattices were measured with a ruler and notated on paper. This sometimes required manipulation of the lattice and multiple measurements of oblong lattices, which required longer times for the lattice to be at room temperature. This may have introduced a new variable. Our updated analysis method provided three improvements. First, the amount of time and lattice manipulation was likely reduced; second, lattice photographs allowed us to measure area rather than diameter, which provides a more precise result; third, a photograph provides a permanent dataset that can be re-analyzed if needed. Even though we reduced lattice size to accommodate the camera and microscope, our contraction results showed a similar pattern as the one in 1992 paper, which is a 40% reduction after 2 minutes and 50% reduction after 10 minutes in the rapid contraction period, followed by a slow tractional force period. After the release of tension, stress fibers gradually disperse and cells start to lose adhesion complexes, followed
by the shape change of cells due to the disassembling of actin microfilaments (Tomasek et al., 2002). Our results support the effectiveness of the updated methods to study mechanisms of fibroblast contraction.

FBS contains both contraction agonists and growth factors to support cell division and contractility (Rayan et al., 1996) (Moulin et al., 1997). When no serum is present, there is little or no contraction, fewer cells, and a cell shape that suggests little or no tension generation. In the presence of serum, more cells are present, and their shape suggests greater tension generation leading to increased contraction. The reduction in cell numbers and rounded cell morphology both contribute to the lack of tension. As a cell-mediated process, the contraction of collagen lattice is correlated with cell numbers (Vaughan et al., 2000) (Rosenfeldt and Grinnell, 2000). The contraction ability was defined in early studies as the cell number required to reduce the surface area of a collagen lattice in half (Steinberg et al., 1980). In our study, cell proliferation was likely reduced in the lattice with the absence of serum compared to cells treated with serum (Rosenfeldt and Grinnell, 2000). The combination of agonist absence, reduced cell number, and inappropriate cell connection to matrix all likely participated in the lack of contraction in FBS-free lattices.

Our results of lattices cultured with various serum concentrations indicate that serum-stimulated collagen lattice contraction is dose-dependent process. Collagen lattices retract less in 1% serum than lattices in more concentrated serum. According to the reported studies, serum is composed of LPA-like lipid growth factors (Grinnell et al., 2010) (Kolodney et al., 1993). Those lipid growth factors can be lysophosphatidic acid (LPA), platelet-derived growth factor (PDGF), or Sphingosine-1-phosphate (S1P) (Ridley et al., 1992; Grinnell, 2003). They all function as agonists in the stimulation of tension-relaxed contraction (Rayan et al., 1996). PDGF promotes the contraction of floating lattices while S1P and LPA are the better agonists in contraction of restrained lattices (Grinnell, 2000). LPA is a phospholipid component released by platelets, which stimulates the contraction by a Rho/Rho-kinase pathway (Rhee et al., 2007). LPA stimulates the formation of stress fibers in a similar way to the contractile activity of FBS (Kolodney et al., 1993). The fibroblast contraction in response to LPA and serum is dose-dependent (Rayan et al., 1996) (Tomasek et al., 1992). S1P is a lysosphingolipid that promotes α-sm actin expression by a rho-kinase pathway to stimulate lattice contraction (Urata et al., 2005). The contraction similarity in the presence of 5% and 10% serum suggests a limit to serum responsiveness or a limit to maximum tension generation. One future goal is to test the hypothesis related to tension generation, and how growth factors or different cell types affect this limit.

The differentiation of myofibroblasts is the crucial step in the wound contraction; it is likely that myofibroblasts participated in our stress-relaxed lattices. α-sm actin is a protein expressed by differentiated myofibroblasts and is the main myofibroblast contractile component (Desmouliere et al., 1993) (Serini et al., 1999). Two important elements, TGF-β1 and ED-A FN also play key roles in the differentiation of myofibroblasts and the generation of contractile force. TGF-β1 first upgrades certain contractile elements, such as stress fibers, vinculin-containing adhesion complexes and fibronectin fibrils in the cytoskeletal system. Then, the generation of myofibroblast contraction is characterized by enhancing the expression of α-sm actin by TGF-β1 (Vaughan et al., 2000). Other reports demonstrate that the expression of α-sm actin is increased by the presence of TGF-β, and reduced when TGF-β is blocked by the addition of TGF-sR (soluble TGFβ-receptor type II) or anti-TGF-β antibodies (Moulin et al., 1997; Hinz et al., 2001b). Many data point out that the promotion of α-sm actin expression is regulated by the interdependence relationship of TGF-β and ED-A FN. ED-AFN is induced by TGF-β while myofibroblast differentiation requires ED-A FN (Serini et al., 1998).

Our update of the stress-relaxed collagen lattice model (Tomasek et al., 1992) provides more accurate data, with the assistance of new computer technology and new insights into serum effects. Our results support the conclusion that the stress-relaxed model is the best model to study the contractile activity of fibroblasts and myofibroblasts. In this research, we also test the stimulation of serum on fibroblast contraction, and the stimulation turns out to be a dose-dependent process. The exact components of serum (FBS) and the mechanisms of stimulation are still unknown. According to the recent studies, we suspect that the serum is a type of agonist, which works in the similar pathway to other growth factors, including LPA and S1P (Grinnell et al., 2010) (Kolodney et al., 1993). We expect to conduct further investigation in this regard, and find the correlation between the serum and other growth factors.
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BIOGRAPHIES
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Potato Catalase Enzyme Kinetics: A Simple Experimental Method

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ABSTRACT
Learning to perform an enzyme kinetics experiment is essential in any experimental biochemistry class. Enzyme catalase has proven to be an excellent choice for teaching simple enzyme kinetics (Deisseroth and Dounce, 1970; Dekock et al., 1979; Johnson, 2000; Kimbrough et al., 1997; Lewis et al., 2009). Catalase is a ubiquitous heme protein that catalyzes the decomposition of H$_2$O$_2$ to water and molecular oxygen (Dekock et al., 1979). This communication describes the modification of an existing kinetic protocol to introduce an easier, more efficient procedure for studying catalase (Johnson, 2000). First, kinetic studies were done in a 5.0 mL polypropylene tube, making the experiment easier to implement and, secondly, only 5 mLs of a hydrogen peroxide solution of different concentrations were utilized for each trial to reduce cost and chemical waste. In addition, spectrophotometers were not required to collect the kinetic data. Soluble proteins containing catalase were isolated from a Russet potato. A small fiberglass filter disc saturated with catalase extract was transferred into a polypropylene tube filled with various concentrations of H$_2$O$_2$. The oxygen produced in this reaction floats the filter to the top of the H$_2$O$_2$ solution. Catalase activity was measured by timing the rise of the enzyme soaked fiber glass filter disc to the top. The data were fitted to the Michaelis-Menten equation and plotted as a Lineweaver-Burk double reciprocal plot from which the kinetic parameters V$_{max}$ and K$_m$ were determined. The concentration of protein on the fiber glass filter was calculated using the Bradford protein assay (Bradford, 1976).

I. INTRODUCTION
Oxygen is the ideal terminal electron acceptor for the electron transport chain. However, partial reduction of oxygen leads to the generation of superoxide anion and hydrogen peroxide. The enzyme superoxide dismutase neutralizes the superoxide anions, converting them into hydrogen peroxide and molecular oxygen. The hydrogen peroxide is then scavenged by catalase, a ubiquitous heme protein that catalyzes the decomposition of H$_2$O$_2$ to water and molecular oxygen, as shown in equation 1 (Johnson, 2000).

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} (l) + \text{O}_2 \]  

(1)

The enzyme catalase is present in animals, plants, and microorganisms (Clayton, 1959; Drory and Woodson, 1992; Esaka and Asahi, 1982; Herbert and Pinsent, 1948; Schonbaum and Chance, 1976). In this communication, soluble proteins containing the enzyme catalase were extracted from a potato and used to study enzyme kinetics.

II. MATERIALS

| 1 medium-sized Russet potato | blender |
| ice-bath container | funnel |
| Fisher brand glass fiber filter circles | hole-punch |
| tweezers | Erlenmeyer flask |
| 5ml polypropylene tubes | stopwatch |
| 1ml and 10ml graduated cylinder | 3% H$_2$O$_2$ |
| Fiber glass filter paper | 0.5 ml micro-centrifuge tubes |
| Spectrophotometer | Bradford reagent |
| BSA (Bovine Serum Albumin) | cuvette |
| pipette | |

III. METHODS

Enzyme Extraction
A medium sized Russet potato was obtained from a local supermarket. The potato was peeled and cut into cubes. The potato cubes were covered with clear plastic film and stored in the refrigerator. A filter device
was set up by placing a small amount of fiber glass at the bottom of a funnel. The potato cubes were homogenized in a blender for 2 minutes. The homogenate was placed in the funnel and the soluble proteins containing catalase were extracted at 4°C using the gravity filtration system shown in Figure 1. The amount of total protein applied to the fiber glass disc was measured using the Bradford reagent and BSA as protein standard.

**Figure 1.** Experimental set-up. A small lump of fiberglass was placed at the bottom of the funnel. A filter paper folded into a cone shape was placed on top of the fiberglass. Ground potato mix was poured onto the filter paper and the protein extract collected. The catalase kinetics experiment was done using a 5.0 ml USA scientific polypropylene tube with a flat cap.

**Enzyme Kinetics Study**

Hydrogen peroxide solutions with concentrations of 0.25, 0.50, 1.0, and 3.0% were prepared from a 3% stock solution. A hole-punch was used to cut a piece of fiber glass filter paper with a diameter of 0.6 mm. Four 5 mL polypropylene tubes were set on a tube stand and 5 mLs H₂O₂ of appropriate concentration were added into each tube. The freshly extracted protein solution containing catalase was diluted with deionized water in 1:5 ratios. Using a micropipette, 5 µl of catalase extract was added onto each of the filter paper discs. A tweezer was used to place the filter paper disc at the bottom of the tube containing the H₂O₂ solution. Occasionally, the filter paper disc may stick to the side of propylene tube. Those data points should be repeated. Time in seconds was recorded from when the filter paper was introduced into the H₂O₂ solution to the moment the filter paper disc reached the surface of the solution. The process was repeated three times for each concentration of hydrogen peroxide and the data recorded. A Lineweaver-Burk plot was generated using the average times, and the V_max and K_m values were determined. The potato catalase concentration may vary from preparation to preparation. A trial run of the highest and lowest hydrogen peroxide concentration will ensure experimental success.

**IV. RESULTS AND DISCUSSION**

Potato catalase is commonly used in biochemistry and biology laboratories for studying enzyme kinetics. This protocol was designed to allow students to easily extract and study the enzyme catalase in a short time and using a small amount of hydrogen peroxide solution and without the use of a spectrophotometer. The experiment results are shown in Table 1. Data were fitted using the Marquardt-Levenberg algorithm (Bishop, 1995) supplied with the EnzFitter program from BIOSOFT, Cambridge, U.K., and the V_max and K_m was determined to be 0.124±0.003 s⁻¹ and 1.17±0.07 mM respectively (Figure 2). Alternatively, Excel’s Solver algorithm may be used to plot V versus [S] data and fit the data to the Michaelis-Menten equation using nonlinear fitting. This experiment has been included in the Experimental Biochemistry classes at the University of Central Oklahoma (UCO) since 2010. The class average for V_max and K_m was observed to be 0.12±0.003 s⁻¹ and 0.98±0.06 mM, respectively.

**Table 1. Enzyme Activity of Potato Catalase**

<table>
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<tr>
<th>H₂O₂ Conc. (%)</th>
<th>Trial 1 Time/s</th>
<th>Trial 2 Time/s</th>
<th>Trial 3 Time/s</th>
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<th>K_m (M)</th>
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<td>11.2</td>
<td>0.089</td>
<td>0.33</td>
<td>11.2</td>
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</tr>
</tbody>
</table>
The Bradford protein assay was done to determine the amount of total protein added to each disc. Based upon this assay, the protein concentration was calculated as 7.5µg of protein/filter.

This experiment has proven to be enjoyable to students. They were able to extract an enzyme and perform kinetic studies on the protein of interest in a few hours. With few exceptions, all the students had reproducible, consistent results. They also learned to use Microsoft excel solver (Kemmer and Keller, 2010) and the EnzFitter program to plot their data set and determine the kinetic parameters $V_{\text{max}}$ and $K_m$.

In this experiment for the first time USA scientific 5.0 ml polypropylene tubes were used to perform the kinetic studies on the enzyme catalase. This method reduces the amount of substrate necessary for the experiment compared to previously published methods (Johnson, 2000; Lewis et al., 2009) and consequently reduces the cost and facilitates the clean-up and disposal of hydrogen peroxide. Spectrophotometers were not required to collect the kinetic data and thus sharing the limited number of spectrophotometers among the students is not necessary. The experiment is easily performed in a teaching lab with many students and each student is able to do their own experiment.

V. DISPOSAL
All solutions can be discarded in the sink with lots of water.

ACKNOWLEDGMENTS
We are grateful to Dr. Paul. Sims for valuable discussions regarding undergraduate research. This research is supported by funds from an Oklahoma State Regents Grant for Higher Education (021606), a P20RR016478 grant from the National Center for Research Resources (NCRR) a component of National Institute of Health (NIH), and grant 011547 from the UCO Office of Research and Grants to L.C.

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BIOGRAPHIES

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Tissue Engineered Polyethylene Glycol Diacrylate (PEGDA) Hydrogels: Effects of Networked Structure, Photo-Initiator Concentration, and Incubation Duration on Cell Viability

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ABSTRACT
Cells embedded deep within the engineered tissue constructs receive inadequate oxygen and nutrients, reducing the functions and life span resulting in improper tissue regeneration. Tissue scaffold engineering requires conduit networks within a biocompatible tissue construct to enable cell survival for prolonged periods. In this study flexible hydrogels, semi-solid water soluble tissue constructs possessing networks and cells, were fabricated with UV photo-polymerization. Fabrication materials included a liquid polymer, polyethylene glycol diacrylate (PEGDA), ultra-violet light activated photo-initiator solvent, and cultured human DP147 mesenchymal fibroblasts, motile skin stem-cells involved in the healing process. UV light activates the photo-polymerization of the PEGDA, encapsulating the cells as curing occurs. Viability experiments analyzed effects of conduit networks, photo-initiator concentration, and cell longevity in cultured hydrogels with a viability assay kit and fluorescent microscopy. Assay results demonstrated networked PEGDA hydrogels possessed increased viability compared to non-networked and decreased viability with increased photo-initiator concentrations and prolonged incubation periods in all hydrogels. Further research using higher molecular weights of PEGDA, improved designs for networked molds, a device for attaining thin uniform assay samples, and the infusion of nutrients in hydrogels could increase the cell viability during incubation.

Key Words: tissue engineering, scaffold, hydrogel, cell viability, polyethylene glycol diacrylate (PEGDA), fibroblasts

I. INTRODUCTION
Tissue engineering is an emerging field that integrates science, engineering, and biology for developing techniques and methods to produce models for natural tissue simulation and for patient tissue and organ implants. A principal challenge in engineering tissues, especially in producing large biocompatible tissue constructs for multiple biomedical applications, including using patient cells for tissue implant, is the cell survivability in constructed tissue scaffolds. Each year, thousands of soft tissue surgeries occur due to trauma and disease (Hutmacher, 2000). Injuries range from light abrasions to severe internal life threatening wounds requiring medical attention. Several researchers developed porous three-dimensional permeable scaffolding, allowing the slow diffusion of oxygen and nutrients necessary for the viability of encapsulated or attached cells using a variety of materials and fabrication methods. The unique ability of networked three dimensional (3D) structures to elicit altered cell behavior including adhesion and growth has raised already heightened interest in scaffold materials for various biomedical applications, including orthopedic and osteopathic tissue repair and regeneration (Lee and Mooney, 2001). In vitro cells, cultured in the lab, usually do not develop in a three dimensional fashion unless allowed to grow on or within a scaffolding construct. Biocompatible scaffolds require specific pore shapes, sizes, and mechanical properties enabling nutrient flow, cell functions, and growth in 3D. Cells, inside the scaffold, must be able to develop, attach, migrate, proliferate, communicate, and differentiate to function similar to human tissues.

Several engineered tissue grafts have been developed for the reconstruction of injured soft tissues (Sun et al., 2004). For example, a polyethylene glycol diacrylate (PEGDA) and sodium alginate hydrogels have been investigated by several researchers as soft tissue grafts (Kim, 2008; Zein, 2002). Hydrogels possess mechanical properties similar to natural body tissue (Shor, 2007). The porous structures permit cell developmental growth, intra/extra cellular communication, cell function, and provisional means for ade-
quate oxygen and nutrients (Gleghorn, 2008). In this research project, PEGDA hydrogel design, fabrication, and cell biocompatibility were investigated and analyzed. PEGDA was selected over other materials for its known biocompatibility, high water absorbance, diffusion characteristics, and application properties for simple design and manufacture of 3D networked hydrogel structures using a UV photo-polymerization process. The photo-initiator (PI) was selected for its biocompatibility and 365 (nm) activation light wavelength. The UV light activates the PI starting PEGDA polymer cross-linking reactions resulting in a flexible gel. UV photo-polymerization is commonly used in micro-fabrication to produce desired scaffolds with high levels of detail and precision. This process has been found to be a valid method to manufacture multi-layer scaffolds allowing the construction of nutrient conduit networks, or channels, within the scaffold to better distribute nutrients and oxygen to the cells (Yasar et al., 2009). This tissue engineering system is comprised of three major parts: 1) UV light source, 2) hydrogel mold and 3) hydrogel cell polymer solution. Hydrogel molds of various materials, including acrylic, aluminum, and silicon polymer, were designed to allow the PEGDA cell hydrogel solution to polymerize with desired shape, size, and architecture. Understanding cell viability in PEGDA constructs with respect to the fabrication process parameters such as networks, photo-initiator concentration, and hydrogel culture time is necessary to adequately design and effectively use PEGDA for tissue engineering constructs. The objectives of this study were two fold. First, to incubate hydrogels at different time periods in 3D PEGDA scaffolds with different internal architectures for nutrient distribution, and varying photo-initiator concentrations. Secondly, to conduct cell viability assays to achieve the best cellular viability while obtaining various successful scaffold structure and internal architectures at body temperature and standard room temperature.

II. MATERIALS AND METHODS

A. Hydrogel Solution

Two solutions with cells were combined to make the final hydrogel solution mix. The first solution was composed of the liquid Polyethylene Glycol Diacrylate (PEGDA), M<sub>n</sub> =700 (mol), chosen for the low cost to develop a method; Sigma-Aldrich, diluted with liquid Dulbecco’s Phosphate Buffer Saline (PBS). The second solution consisted of a solute solid photo-initiator (PI) Alpha-alpha-dimethoxy-alpha-phenyl-acetophenone, M<sub>n</sub> =256.35 (g/mol); Sigma-Aldrich, which was dissolved in the liquid solvent 1-vinyl-2-pyrrolidone, M<sub>n</sub> =111.14 (g/mol); Fluka. The two hydrogel solutions were kept in separate aliquots until cells were added just before curing. The PBS volume is adjusted in the first solution to account for the three tested concentrations of the second solution.

B. Cell Culture

DP147 dermal fibroblast cells, used in hydrogels, were acquired using techniques and protocols for culture and isolation. Cells used for culture were incubated at standard conditions, 37 degrees Celsius and 5% CO<sub>2</sub>, in tissue culture dishes with nutrient media, DMEM containing 10% fetal bovine serum (FBS) and 1% anti-bacterial/anti-microbial (ABAM). Cells for hydrogels were isolated by removing nutrient media, washing with DPBS, and adding trypsin to break up cell tissues and suspend in media for counting. Suspended cells were counted three times with a hemocytometer and light microscope and averaged. After counting the average number of cells per volume the current population density was calculated. Cell suspensions were centrifuged for 5 minutes until cell pellets formed, separating cells from the media. Liquid was suctioned from the cell pellet in a test tube followed by adding the two hydrogel solutions and mixing thoroughly directly before UV curing (Vaughan et al., 2004).

C. PEGDA Hydrogel Mold

A sterile open-ended cylindrical borosilicate glass tube, with open end placed on a silicon rubber disc functioned as a mold for cylindrical shaped hydrogels. In addition, the glass functioned as hydrogel housing that could be turned over providing nutrient media surface contact for the open ends and provided a novel way to acquire thin hydrogel samples for viability assays. To create networked channeled PEGDA hydrogels, thin stainless steel pins were carefully placed into the open glass end and secured into the silicon disc (Figure 2).

D. Specimen Preparation

Figure 1 shows the schematic diagram of the hydrogel specimen preparation for channeled internal structure. Cultured human DP147 fibroblasts, for hydrogel seeding, were trypsinized and counted to add to hydrogel solutions. The 20% PEGDA solution was produced by mixing 2ml of PEGDA with 8 ml of DPBS.
The PI solution was produced by mixing 0.3 (g) of PI powdered solid in 1 (ml) of the liquid vinyl solvent in a dark room to prevent premature cross-linked curing from light. The 0.2%, 0.6%, and 1.0% PI volume concentration hydrogel solutions were produced by adding 4, 12, and 20 (µl) of PI solution with 2 (ml) of PEGDA solution, respectively. The desired hydrogel mixtures were added to the cell pellet and vortexed to ensure thorough mixing. For curing, a 365 nanometer (nm) UV lamp was used to photo-polymerize and cell infuse the hydrogel. An adjustable multi-level stage designed to support the UV-lamp and hold hydrogel was built and implemented to adjust the light intensity. The lamp was turned on 20 minutes before hydrogel curing to reach maximum UV light intensity.

Under the aseptic conditions of a biological safety cabinet hood, custom made molds, for networked and non-networked hydrogels (see Figure 1) were placed in covered 35 (mm) tissue dishes for sterile curing. Hydrogel cell solutions were pipetted into a mold and cured in layers under the lamp. Excess liquid was removed under the hood after each layer cured, and the following layers were added and photo-polymerized. For networked hydrogels, caution was taken during removal of secured pins so as not to damage the delicate structures. Finished hydrogel samples were rinsed twice in DPBS to remove the non-cured hydrogel liquid solution. Next cured hydrogels were directly placed in new tissue culture dishes containing nutrient media and incubated at standard conditions of 37 Celsius and 5% CO₂. Nutrient media was removed and replenished every three days during the incubation period.

**Figure 1** Schematic diagram for the hydrogel specimen preparation.

**E. Viability Assay**

Viability of cells infused in PEGDA hydrogels was assessed using the fluorescent microscopy and the LIVE/DEAD Viability/Cytotoxicity Kit, for mammalian cells (Molecular Probes, Invitrogen). Protocol recommended by Invitrogen for this assay was followed. The example dilution protocol supplied by Invitrogen was followed. The LIVE/DEAD 10 (ml) solution consisted of sterile DPBS and the two supplied kit stock solution, 20 (µl) of EthD-1, µM/solution and 0.4 (µl) calcien AM 2, µM/solution.

**F. Measurement of Cell Viability**

Viability of cells infused into PEGDA hydrogels was assessed with Molecular Probes, Invitrogen LIVE/DEAD Viability/Cytotoxicity Kit, for mammalian cells. Protocols supplied with this kit and recommended by Invitrogen were followed for this assay study. The kit provided two different colored fluorescent probes that simultaneously mark live and dead cells to measure cell viability. The probes recognize intracellular esterase activity and integrity of the cell membrane. Fluorescent microscopy was used to detect the probes and determine cell viability. Two probes, calcien AM and Ethidium homodimer-1 (EthD-1) were used in the assay. EthD-1 enters damaged membranes of dead cells and binds to nucleic acid producing a bright red fluorescence at wavelengths of 495 (nm) to 635 (nm). EthD-1 is non-permeable to a healthy intact cellular plasma membrane. Calcein AM, the non-fluorescent form, is converted to calcein by enzymes. The converted probe, calcein, produces an intense green fluorescence in live cells at range from 495 (nm) to 515 (nm). Both probes are virtually non-fluorescent until cellular interactions occur, reducing background fluorescence error in cell viability quantification.

Invitrogen fluorescence microscopy protocol for the LIVE/DEAD assay was followed. Optical filters were selected for optimum observance of calcein and EthD-1. Two different band-pass filters were chosen for the individual probes resulting in two fluorescent images, red and green. A digital camera, attached to the UV microscope, and computer imaging software captured, saved, and merged the (10x) magnified images. The final third image, a combined red and green, consisted of two merged saved images. Viability was calculated from the merged images by counting the number of live green cells and dead red cells. The equation for hydrogel cell viability, (number of live cells) *100%/(number live and dead cells)), was applied to each merged image. Multiple assay samples were collected from each
hydrogel during culture to show the percent change in cell viability over the hydrogel incubation period.

The LIVE/DEAD viability assay solution was first applied to small samples of the suspended cells before adding to the hydrogel solution. After the curing, thin sample sections of the incubated hydrogel were collected for hydrogel cell viability at (days) 1, 3, 5, and 7. Borosilicate glass tubes, placed on silicon polymer, functioned as a mold for cylindrical shaped hydrogels. In addition, the glass functioned as hydrogel housing that could be turned over, exposing both open ends, providing nutrient media surface contact and a novel way to acquire thin hydrogel samples for viability assays.

The sectioning of cell imbedded hydrogel specimen samples, enclosed in glass tube housing for separated slices by scalpel, for analysis of hydrogel cell viability is illustrated in Figure 2. Thin even sections ranging from 0.5-1.0 (mm) were sampled from the incubated hydrogels with difficulty. Acquiring desired sample sections with certain dimensions for the viability without damaging the hydrogel structure was a difficult task. This process was improved by designing a prototype device to hold the hydrogel in the glass tube securely. Once the glass was secured the built-in micrometer could be turned to move the hydrogel out of the glass housing in desired increments for samples. A scalpel was used to section thin disks from the hydrogel. After sectioning the sampled hydrogel was rinsed with DPBS and placed back in normal incubation conditions. Sample sections were rinsed twice with DPBS to remove media. Next, the viability assay LIVE/DEAD solution was pipetted onto sample sections, in 35 (mm) tissue culture dishes. Sampled sections were covered with aluminum foil and incubated for 75 minutes. After the incubation period, sections were rinsed twice with DPBS, to remove excess stain, and placed on microscope slides emerged in DPBS to prevent dehydration and remove unwanted liquids. Samples were assayed and viewed with two fluorescent microscope filters to produce an image for viability analysis.

**Figure 2** Sectioning of hydrogel specimen for cell viability experiments. (a) The cured cell infused hydrogel is placed in a custom made holder equipped with micrometer. (b) The micrometer allows for small increments of PEGDA hydrogel to be pushed outside from the tube to be sliced. (c) Thin hydrogel slices are obtained to later analyze under the microscope. (d) Tissue culture dish with two borosilicate glass hydrogel molds on silicon disc and steel pins for networks.

### III. RESULTS AND DISCUSSION

Cells were successfully grown in UV cross-linked PEGDA hydrogel structures with significant viability differences in networked architecture and varying concentrations of the photo-initiator as shown in Figure 3. Table 1, Table 2, and Figure 4 illustrate the collection of cell viability data for four different experiments. The cell viability parameters considered were the hydrogel culture incubation time length and the photo-initiator concentrations. The times selected were pre-cure, which represents the viability prior to infusing the cells in PEGDA constructs, and post-cure, 24 hour durations after cure, three days, five days, seven days, one week, and lastly two weeks. The ideal biocompatible concentration of PI for cell viability in constructs was found to be 0.2%.
Figure 3 Cell Viability pictures taken under fluorescence microscope after 24 hours incubation: (a) 20% PEGDA with 0.2% P.I., (b) 20% PEGDA with 0.6% P.I., and (c) 20% PEGDA with 1% P.I. Green stained cells are living cells in 0.2% photo initiator hydrogel. Red stained cells are dead cells when photo initiator concentration is 0.6%. At 1.0% photo initiator concentration more dead cells are observed compared to living cells.

Table 1. Data table for cell viability plots for channeled structures

<table>
<thead>
<tr>
<th>P.I. concentration (%)</th>
<th>Cell culture time (days)</th>
<th>Cell density</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>Pre-Cure</td>
<td>97 ± 1.82</td>
</tr>
<tr>
<td>0.2</td>
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<td>57.3 ± 3.37</td>
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<td>0.2</td>
<td>3</td>
<td>46.1 ± 6.65</td>
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<td>0.2</td>
<td>5</td>
<td>39.7 ± 8.18</td>
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<td>0.2</td>
<td>7</td>
<td>32.7 ± 3.52</td>
</tr>
<tr>
<td>0.6</td>
<td>Pre-Cure</td>
<td>99 ± 0.82</td>
</tr>
<tr>
<td>0.6</td>
<td>1</td>
<td>43.5 ± 2.91</td>
</tr>
<tr>
<td>0.6</td>
<td>3</td>
<td>36.2 ± 2.90</td>
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<tr>
<td>0.6</td>
<td>5</td>
<td>33.5 ± 3.83</td>
</tr>
<tr>
<td>0.6</td>
<td>7</td>
<td>28.2 ± 5.14</td>
</tr>
<tr>
<td>1.0</td>
<td>Pre-Cure</td>
<td>98 ± 0.82</td>
</tr>
<tr>
<td>1.0</td>
<td>1</td>
<td>34.8 ± 5.31</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>27.8 ± 2.14</td>
</tr>
<tr>
<td>1.0</td>
<td>5</td>
<td>26.5 ± 1.68</td>
</tr>
<tr>
<td>1.0</td>
<td>7</td>
<td>20.4 ± 5.97</td>
</tr>
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</table>

Table 2. Data table for cell viability plots for Non-channeled structures.

<table>
<thead>
<tr>
<th>P.I. concentration (%)</th>
<th>Cell culture time (days)</th>
<th>Cell density</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>Pre-Cure</td>
<td>97 ± 0.82</td>
</tr>
<tr>
<td>0.2</td>
<td>1</td>
<td>54.6 ± 3.11</td>
</tr>
<tr>
<td>0.2</td>
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<td>7</td>
<td>27.5 ± 1.41</td>
</tr>
<tr>
<td>0.6</td>
<td>Pre-Cure</td>
<td>98 ± 0.82</td>
</tr>
<tr>
<td>0.6</td>
<td>1</td>
<td>42.2 ± 3.41</td>
</tr>
<tr>
<td>0.6</td>
<td>3</td>
<td>39.4 ± 1.65</td>
</tr>
<tr>
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<td>5</td>
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<td>7</td>
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<tr>
<td>1.0</td>
<td>Pre-Cure</td>
<td>99 ± 0.82</td>
</tr>
<tr>
<td>1.0</td>
<td>1</td>
<td>35.4 ± 4.57</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>25.1 ± 1.66</td>
</tr>
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<td>1.0</td>
<td>5</td>
<td>24.3 ± 2.31</td>
</tr>
<tr>
<td>1.0</td>
<td>7</td>
<td>17.5 ± 1.33</td>
</tr>
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</table>

Figure 4 Chart for cell viability results for 20% PEGDA hydrogel constructs.

No statistical significant difference of $P$-values was found between channel and without channel PEGDA samples for different PI concentration and incubation time samples ($P$ values $\geq 0.05$). A statistically significant differences in cell viability between 0.2% and 0.6% PI
concentration PEGDA samples were found after 3 and 5 days of incubation times for both channel and w/o channel PEGDA samples ($P$ values <0.05). Additionally, statistically significant differences in cell viability between 0.6% and 1% PI concentration PEGDA samples were found after 3 and 5 days of incubation times for both channel and w/o channel PEGDA samples ($P$ values <0.05). Statistically significant differences in cell viability between pre-cure and 3 days incubation times PEGDA samples were found for 0.2%, 0.6% and 1% PI concentration PEGDA samples ($P$ values <0.05). Statistically significant differences in cell viability between 3 and 5 days incubation times PEGDA samples were found for 0.2%, 0.6% and 1% photo initiator concentration PEGDA samples ($P$ values <0.05).

Viability results were not as high as expected when compared to 80% fibroblast viability at 14 days observed by other research (Hutmacher, 2000; Lee and Mooney, 2001). Channels exposing cells to nutrient flow should demonstrate increased viability. The observed shorter than expected life span of cells over culture time could result from many factors. High heat and/or UV light intensity in the curing process could cause damage to cells and functions. A device for more accurate and precise samples needs to be implemented or the current version sample collector created could be remodeled to yield thinner samples for better analysis using fluorescent microscopy. Variations on curing times and UV intensity, adding nutrients to hydrogel solution, increasing the cell seeding density, substituting higher molecular weights of PEGDA could improve the viability as noted by the previous authors. Substituting higher molecular weights of PEGDA could result in better suited structures for nutrient diffusion and cell motility. Cell integration for tissues requires the following for needed viability and proliferation, the ability to migrate through hydrogel by remodeling the construct or by hydrogel material degradation, the proximity location to neighboring cells for cellular communication, and a flexible or shapeable space for cell development, growth and differentiation.

IV. CONCLUSION

In this work the UV photo-polymerization system has been successfully developed to design and fabricate networked PEGDA hydrogel structures. An extensive cell viability assessment of the PEGDA hydrogels was conducted in this study to relate the function of the network architectures, photo-initiator concentration, and cell infused hydrogel incubation culture time. Further analysis of hydrogel viability is required to find the most suitable engineered tissue for implant tissue.

ACKNOWLEDGEMENTS

This work is the result of research activities supported by the UCO Department of Engineering and Physics and the UCO Office of Research and Grants. The authors would like to thank Dr. Ozlem Yasar, Assistant Professor, New York City College of Technology (NYCCT) for her help in the PEGDA tissue preparation and also acknowledge the contributions of classmates Albert Orock and Stefano Tarantini.

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**BIOGRAPHIES**

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Use of Dragon’s Blood (*Daemonorops draco*) Pigment in Photovoltaic Cells

Brett A. Jones and James E. Bidlack
Department of Biology

ABSTRACT
An experiment was conducted in an environmental chamber with artificial lighting to determine the viability of a plant pigment known as Dragon’s Blood (*Daemonorops draco*) for use in dye-sensitized photovoltaic cells. Upon exposure to light, these cells produced mean voltage, current, and power readings of 150 mV, 1.68 microamps, and 0.289 microwatts, respectively, for a period of nineteen days. These readings were significantly higher than values obtained at night and substantially higher than values obtained from cells constructed without pigment. The low cost of constructing these cells, coupled with their longevity, suggests that they have potential as economically-feasible and sustainable energy alternatives.

I. INTRODUCTION
One of the most promising alternative energy technologies for traditional fossil fuel technology is the photovoltaic cell, which converts light energy into clean, renewable energy in the form of electricity. However, traditional photovoltaic cells built with silicon require expensive, specialized equipment to construct; the offset of this drawback is that after construction, the maintenance costs are minimal and fuel costs are nonexistent. An alternative to the common silicon photovoltaic cell exists in the form of thin film photovoltaic cells (O’Regan and Gratzel, 1991). These cells are constructed by applying one or more photovoltaic layers onto a thin semiconductor material. While not as efficient as traditional silicon photovoltaic cells (Carlson and Wronski, 1976), thin film photovoltaic cells are less expensive to produce and have a wider potential range of application.

A subset of the thin film photovoltaic cells are dye-sensitized solar cells (DSSCs), which are comparable to the efficiency of thin film photovoltaic cells (Jayaweera et al., 2008), but are easier and cheaper to construct (Smestad and Gratzel, 1998). However, there are challenges encountered with stability of these cells over time. Currently, there are some DSSCs used in commercial products which use inorganic dyes to help overcome these issues. To make this technology viable as a candidate for replacing fossil fuels, the costs of production must be low, because the power produced by each DSSC is also quite low. The manufacturing methods should be relatively simple and the materials used must be inexpensive and plentiful. While cells constructed with ruthenium-based dyes (along with a platinum counter-electrode) are simple to manufacture and fairly efficient, these materials are scarce and thus expensive, which offsets the benefits of DSSCs (Gratzel, 2005). Ruthenium is also toxic, which requires more care in handling.

Organic dyes (water soluble), as well as pigments (water insoluble), are attractive alternatives because they are more available and often inexpensive, but the stability of cells constructed with such substances remains a problem. Photosynthetic pigments (carotenoids and chlorophylls), for instance, have much potential for use in DSSCs, but these substances are not very stable and often oxidize if not treated properly after extraction. In this experiment, an ancient pigment known as Dragon’s Blood was evaluated to determine its efficiency and stability as part of DSSCs because it is readily-available and retains its color for months, years, and even decades following extraction. This pigment is derived from *Daemonorops draco* (Blume) and has been used in various applications since antiquity, including scenarios where it has proven to be durable even when exposed to sunlight (Edward et al., 2001; Gupta et al., 2008).

II. MATERIALS AND METHODS
Photovoltaic cells were constructed and tested in the Biology Department at the University of Central Oklahoma. Procedures for constructing these cells were derived from a Nanocrystalline Solar Kit purchased from the Institute of Chemical Education at the University of Wisconsin in Madison, Wisconsin.
**Use of Dragon’s Blood (Daemonorops draco) Pigment in Photovoltaic Cells**

Conductive glass was obtained from Hartford Glass Company of Hartford City, Indiana. Titanium dioxide was purchased from Degussa USA of Akron, Ohio. Elmer’s Superglue® was purchased locally. The resin required to make the Dragon’s Blood \textit{(Daemonorops draco (Blume))} pigment was obtained from Mountain Rose Herbs of Eugene, Oregon. All other materials were supplied from Sigma-Aldrich in St. Louis, Missouri. An ADC-16 board connected to a data logger from Pico Technology, Ltd., United Kingdom, was used to monitor and record voltages produced by the photovoltaic cells.

Exactly 5.0 grams of Dragon’s Blood resin was crushed into powder and soaked in 40 mL of acetone for 72 hours to create the Dragon’s Blood pigment preparation. A large amount of residue remained, which was discarded, and the resulting supernatant was placed in four separate 15 mL centrifuge tubes. These tubes were centrifuged at 3,000 rpm for five minutes to pellet any remaining residue and the final supernatant in each of the tubes was combined. This extract, which visually appeared to retain a deep red color for many months after preparation, was used within two weeks for making the photovoltaic cells.

Test cells were constructed using two glass plates coated with tin oxide for conductivity. Conductivity of the glass plates was measured prior to construction by using a Volt-Ohm meter and indicated a coating resistance that ranged from 22 to 23 Ohms. The semiconductor, titanium dioxide (TiO$_2$), was prepared by dissolving approximately 6.0 g of TiO$_2$ in 10 mL of acetic acid, and grinding with mortar and pestle for 30 minutes. A thin layer of this TiO$_2$ suspension, applied with a glass rod, was annealed to the conductive surface of the anode using a Bunsen burner, and left to cool for fifteen minutes. The final surface area of the TiO$_2$ layer in each cell was 1.0 square centimeter. Each test anode was then soaked in a beaker with the pigment preparation for 72 hours to ensure that the pigment was embedded in the TiO$_2$ layer. The resulting treated anodes were then left to dry for 24 hours. Control cells were not left to soak in the pigment preparation and thus remained as TiO$_2$ without pigment.

The conductive side of the cathode for each cell was coated with graphite, and each cathode was then placed with the graphite facing the TiO$_2$ coated side of the anode. A KI/I$_2$ electrolyte solution, which contained 50% saturated KI and 50% saturated I$_2$, was then injected between the opposing plates. The cell was completed by sealing the glass plates together with Elmer’s Superglue® and allowing the finished cell to dry for 24 hours. Any noticeable leakage was sealed, and the cells were then used for the experiment. The experiment took place in an environmental chamber where light was supplied by four 39 Watt Philips F39T5/841 high output bulbs. Light intensity ranged from 20,000 to 30,000 lumens (approximately 40 watts per square meter) during a 12-hour day period to near-zero lumens during a 12-hour night period. Power curves, obtained by plotting current versus voltage were constructed for all cells using a variable resistor with values from 0 to 10,000,000 Ohms. It was determined that the maximum power value for these cells was obtained at about 100,000 Ohms. Hence, a resistor with a value of 100,000 Ohms was inserted, in parallel, for each cell in order to obtain current and power readings (using Ohm’s Law) throughout the experiment. Eight cells were constructed for this experiment, which included four controls and four treatments. The photovoltaic cells were arranged in the chamber as a randomized complete block design with four replications. Voltage measurements were recorded, using Pico Technology software, every 10 minutes for nineteen days.

**III. RESULTS AND DISCUSSION**

Voltage values of all photovoltaic cells during the day were substantially higher than voltage values during the night (Figure 1), confirming that these cells responded positively to light and hence performed as expected. There was an obvious increase in voltage of DSSCs after exposure to light which reinforced the observation that these cells performed as expected. As a night cycle began, the voltage of DSSCs dropped to minimal values, but this decrease was not immediate, which accounted for some positive voltage readings even in the absence of light. For cells treated with Dragon’s Blood pigment, voltages peaked at 230 mV during the day and 77.5 mV at night, whereas voltages of control cells peaked at 30.0 mV during the day and 2.82 mV at night. Photovoltaic cells with pigment achieved much higher voltage values than control cells during the day, and somewhat higher voltage values than control cells at night, suggesting that the pigment embedded in TiO$_2$ operated as expected to absorb light energy.
Figure 1. Voltage readings of photovoltaic cells made with and without Dragon’s Blood pigment during the day and night over a nineteen day period. Standard errors are shown for each point, which represents an average of four replications, each of which represents readings of cells taken every 10 minutes.

Peak voltage of all cells was obtained after about five days in the environmental chamber, and declined thereafter. However, voltage readings during the day did not decline as rapidly in cells treated with Dragon’s Blood pigment. In fact, the pigment-treated cells maintained about the same reading of about 150 mV that was obtained at the beginning of the experiment, despite the peak of 230 mV at five days. Control cells, on the other hand, showed a drop in voltage output during the day and night after the fifth day peak, and the pigmented cells at night reported very little voltage.

Although the experiment was planned to last at least a month, the campus experienced a snowstorm and lost power for several days. As such, the lights and the battery backup for the equipment monitoring the experiment stopped functioning and the experiment ended. By the end of the last recorded day, day nineteen, voltages for the pigmented cells during the day were about 10 times higher than these same pigmented cells at night and more than 10 times higher than control cells during day and night.

The peak and average voltage readings of 230 mV and 150 mV, respectively, for these cells made with Dragon’s Blood are within the range of values reported in previous investigations where crude chlorophyll-treated cells achieved a peak voltage of 230 mV and an average daily voltage of 46.1 mV during a ten day period (FitzSimons, 2010). These values are also similar to those reported in the literature for a specially-made chlorine-e6 chlorophyll derivative used in cells that provided a peak voltage of 247 mV (Amao and Komori, 2004). However, none of these voltages are as high as the 455 mV value reported for cells produced from ruthenium-based dyes (Gratzel, 2005).

Current (Figure 2) and power (Figure 3) readings obtained during the day demonstrated the same trends obtained for voltage readings. For photovoltaic cells treated with pigment, current ranged from 1.36 to 2.31 microamps with a daily average of 1.68 microamps, whereas current ranged from 0.086 to 0.290 microamps with a daily average of 0.179 microamps in control cells. Overall, current values for photovoltaic cells treated with pigment were higher and more stable than these same values for control cells. The same trend was observed for power values, in that photovoltaic cells treated with pigment ranged from 0.184 to 0.531 microwatts with a daily average of 0.289 microwatts; whereas power values from control cells were generally negligible with values of less than 0.00774 microwatts for the duration of the experiment. These results suggest that photovoltaic cells constructed with Dragon’s Blood pigment can produce power, although small, and that absence of pigment in photovoltaic cells produces essentially no power.

Figure 2. Current readings of photovoltaic cells made with and without Dragon’s Blood pigment over a nineteen day period. Standard errors are shown for each point, which represents an average of four replications, each of which represents readings of cells taken every 10 minutes.
Figure 3. Power readings of photovoltaic cells made with and without Dragon’s Blood pigment over a nineteen day period. Standard errors are shown for each point, which represents an average of four replications, each of which represents readings of cells taken every 10 minutes.

The peak and average currents of 2.31 and 1.68 microamps, respectively, for these cells made with Dragon’s Blood, are below the range of values reported in previous investigations where crude chlorophyll-treated cells achieved a peak average current of 16.1 and 2.87 microamps, respectively (FitzSimons, 2010). Moreover, all of these values are below those reported in the literature for a specially-made chlorine-e6 chlorophyll derivative used in cells that provided a peak power of 240 microwatts (Amao and Komori, 2004), and substantially lower than the peak current of 9,400 microamps produced from ruthenium-based dyes (Gratzel, 2005). The peak power value of 0.531 microwatts for these cells made with Dragon’s Blood cells was also below the peak power value of 3.74 microwatts for chlorophyll-treated cells, but the average power of 0.289 microwatts for Dragon’s Blood cells was about twice the average daily power value of 0.134 microwatts for the chlorophyll-treated cells (FitzSimons, 2010). The only power value comparison in the literature for similar cells is for the specially-made chlorine-e6 chlorophyll derivatives used in cells that provided a peak power of 59.3 microwatts (Amao and Komori, 2004); which is more than 100 times the value obtained from cells made with Dragon’s Blood.

Results from this investigation demonstrated that photovoltaic cells produced with Dragon’s Blood pigment have potential to produce average daily voltage (150 mV), current (1.68 microamps), and power (0.289 microwatts) values when cells are exposed to light. These values are generally stable, even after nineteen days, suggesting that this type of technology can provide energy from light that may be sustainable. While these cells may not be as productive as some of the more sophisticated DSSCs in the literature, they still exhibit measurable voltage, current, and power. Given the low expense of these cells and the crude nature in which they were made for this experiment, there is potential for keeping the cost minimal for this type of technology and improving photovoltaic cell performance to obtain alternative and sustainable energy. This experiment has inspired future investigations of photovoltaic cells similar to those made with chlorophyll derivatives (Wang et al., 2010) and use of pentacene layering (Chao et al., 2011) to enhance light energy conversion.

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**BIOGRAPHIES**

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