Periodontal Monitoring Device

Senior Project I

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Abstract

Periodontitis is an inflammatory disease that affects the periodontium and results in loss of tissue and/or bone. Current approaches for diagnosing periodontitis in patients involve invasive procedures, such as probing, which can be time-consuming and painful for the patient. While previous studies have utilized gingival crevicular fluid to evaluate periodontal disease, this Periodontal Monitoring Device measures the activity of a salivary biomarker, α-amylase, and correlates it to the progression of periodontal disease. Since this device will be non-invasive and time-efficient, health-care professionals will be able to use this device during multiple stages of treatment for either monthly screenings or post-treatment monitoring. Simulated saliva samples will be used to verify the accuracy of measurements made by the device and to ensure the correct classification of the sample as having mild, moderate, or severe periodontitis.

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Nomenclature

U: 1 (activity) unit. This has various definitions according to the source, including the amount of α-amylase that will liberate 1 mg of maltose for 1 minute at 20 °C[4], or the amount of α-amylase that will liberate 1 μmol of maltose for 1 minute at 25 °C (Sigma-Aldrich).

Introduction

Gingivitis and periodontitis are chronic inflammatory diseases that are considered some of the most prevalent diseases in the adult population. Plaque that stays on or around the teeth longer than two or three days hardens and develops into the tartar, which acts as a reservoir for bacteria to develop and causes inflammation of the gums around the base of the teeth. This initial stage of gum inflammation that precedes gum disease is known as gingivitis. As the inflammation worsens, pockets filled with plaque, tartar, and bacteria between the gums and teeth begin to develop and eventually, advance under the gum tissue. This stage of deep infection is referred to as periodontitis,[1] which can cause bleeding, tooth decay, and eventually bone loss, and affects 15-20% of adults globally.[2] Although inflammation in periodontitis presents redness and swollen gums, the pain, which is often experienced when inflammation occurs in other areas of the human body, is missing from the clinical picture associated with periodontitis.[2] This absence of pain is commonly one of the main reasons why patients do not seek dental care in the earlier stages, allowing the disease to progress to a point at which it requires extensive treatment or it is no longer reversible.

Currently, periodontal disease has been diagnosed and monitored through the conventional dental probing procedure, in which a metal probe is inserted in the spaces between the gum and the teeth to measure the pocket depth. Bleeding on the dental probe is also commonly used to predict periodontal disease activity, but it is only a binary measurement of disease activity and does not provide much information on the level of the disease to health care professional, leading to overtreatment.[3] In addition, both of these probing techniques are known to be very time-consuming for the dentist and painful for the patient.

This device will utilize a simple, conventional colorimetric assay for biomarker and correlate the concentration of the biomarker to disease severity. A biomarker is defined as a measurable substance that is indicative of a disease. Several biomarkers of periodontitis have been found, both in the gingival crevicular fluid (GCF)[4] and saliva[5]. Because collecting saliva is a noninvasive process and because saliva is an easily accessible biological fluid, the use of a salivary biomarker was more favorable for the purpose of our device. Existing devices have been designed as microfluidic chip-based immunoassays, which detect the presence of various periodontitis biomarkers including immune-related proteins and bacterial pathogens, and have only given binary outputs, but none have utilized specific biomarkers to indicate the progression of periodontal disease. With this objective in mind, it is predicted that a point-of-care salivary test device could play an essential role in monitoring the progression of periodontal disease for patients that have already been diagnosed with periodontitis.

Team Management

Team Leader & Webmaster: Sindhuja Kuchibhatla
The role of the team leader is to provide a vision of the project objectives, oversee the design requirements, development process, techniques, and tools being used for the design of the device. The team leader also serves as a meeting manager to ensure that all meetings run smoothly and communicates the team status, tasks accomplished, and next plans of action to the project advisor. The Webmaster manages the team website by providing project information and maintaining an inventory of meeting minutes and design updates.

Budget & Timeline Manager: Naina Iyengar
The role of budget/timeline manager involves creating and maintaining the project's budget by updating the materials list and setting milestones with a Gantt chart.

Record Keeper: Lindsey Cabanas
The role of the record keeper is to keep accurate records of what is occurring during meetings.

Chapter 1: Background

Periodontitis, or gum disease, is an inflammatory condition caused by bacterial infection of the gums, and results from genetics, poor oral hygiene, and consumption of sugary and processed foods/beverages, smoking, or a combination of these. It can be recognized during regular, bi-annual dental visits by its visual symptoms, such as swollen, red gums, which bleed. To differentiate it from gingivitis, a precursor to the disease, and classify the severity (mild, moderate, or severe) dental practitioners will then insert a pointed metal probe in the spaces between the gum and teeth to measure clinical parameters such as pocket depth (PD), bleeding on probing (BOP), clinical attachment loss (CAL), plaque index (PI), and gingival index (PI). If the disease is in the earlier stages and no bone loss has occurred, dentists will then perform scaling and root planing (SRP) therapy, by scraping away plaque on all affected surfaces (disease tooth planes and below the gum line); antibiotic rinses or oral medications may be recommended as well. If bone loss has occurred, or the patient responds poorly to earlier SRP/antibiotics, the only option is to perform flap surgery to remove bacterial deposits well below the gum line, and place bone/tissue grafts to replace the diseased periodontal tissues, ligaments, and supporting alveolar bone. These surgical procedures have associated risks of further infection, and are very expensive for the patient. Furthermore, even after the patient has undergone treatment, patients must be closely monitored every 3 months during periodontal maintenance visits, in which the mentioned clinical parameters must be re-measured to characterize disease progression. This is essential since once initiated, progression of periodontal disease is very unpredictable and occurs in random bursts within a relatively small time frame[6].

An existing device used to diagnose periodontitis is the portable microfluidic device to detect periodontal disease in saliva[7]. This device incorporates a lab-on-a-chip immunoassay to measure periodontal biomarkers such as tumor necrosis factor alpha, interleukin 6, and C-reactive protein to predict the onset of periodontitis. However, this detection method provides only binary results and does not relay information about the severity level or progression of the disease to the user. Another existing device in the periodontal field is the MyPerioPath, which is used as a salivary diagnostic tool, to give concentrations of specific perio-pathogenic bacteria[8]. Like the microfluidic chip technique, MyPerioPath does not provide information about the severity of the disease either. Finally, though it is still under development, a salivary test from Columbia University uses β-glucuronidase as a biomarker to detect periodontal disease[9].

Since the mentioned clinical parameters must be measured for each tooth, periodontal maintenance visits are very time-consuming: the average appointment lasts about 1:16:23 $\pm$ 0:19:15 hours[10]. The associated probing procedure is invasive, and potentially uncomfortable or even painful for the patient - a preliminary study found that 20-33% of patients experience significant pain during periodontal maintenance visits[11]. This device will require the collection of saliva, which is non-invasive and non-painful, and will utilize a rapid (~1 min.), colorimetric, chemical assay for a salivary biomarker for disease severity, eliminating the above problems with current periodontal maintenance visits. Furthermore, this device will feature step-by-step instructions for a chemical protocol, which requires minimal pre-mixing of widely available and relatively cheap reagents (included in the device "kit") ensuring that personnel with minimal health and science training can use it. Therefore, the intended users for this device include not

only dental professionals within an office setting to minimize time-consumption and patient pain, but also volunteers in medically underserved areas such as those serviced by mobile dental clinics and volunteers/locals in developing countries.

Chapter 2: Chemical Aspect (Naina Iyengar)

One salivary biomarker that has recently been found for periodontitis is α-amylase.[12] α-amylase is found naturally in saliva, however, it has been found to increase as periodontal disease progresses (from healthy to mild, moderate, or even severe) since it is involved in non-immunological defense in the oral cavity including mucosal integrity[13], including growth inhibitory activity against *P. gingivalis* and interference with *A. actinomycetemcomitans* aggregation/biofilm formation, both periodontal pathogens.[14] It is important to note that salivary α-amylase can increase with other conditions which affect the salivary gland, so there will be a few exclusion criteria for the patient base of this device.

Starch is an energy-storage polysaccharide produced by plants which is mixture of amylose (linear portion) and amylopectin (branched portion). When added to a potassium iodide-iodine solution (a solution of elemental and ionic iodine), a blue-black starch-iodine complex is formed. It is speculated that it does this as I_5^- molecules form a linear arrangement within the coils of β-amylose, causing a transfer of charge between them and a subsequent change in the energy level spacing of the iodine so that it can absorb visible light to produce the said color.[15] However, if α-amylase is added, which can break the α(1→4) bonds of α-amylose to produce maltose, a disaccharide of α-D-glucose, there will be less starch available to fix the iodine, resulting in a change of color back towards natural in iodine (clear/yellow). This shift in absorbance is detectable with a spectrophotometer, and so related principles of comparing incident and transmitted light in a starch-iodine solution are one way of measuring the presence and amount of α-amylase in a sample. It is important to note the definition of Beer's Law: $A=\varepsilon bC$, where A is the absorbance (fraction of emitted light relative to the incident light), ε is the molar absorptivity of the solution, b is the path length of the cuvette, and C is the concentration; standard curves plot the absorbance on the y-axis and the concentration on the x-axis at a particular wavelength.

In this project, for measuring and economic efficiency, the device will be designed around, and tested with, α-amylase from *Aspergillus oryzae*, a filamentous fungus historically used to ferment soy and bean curd in Japanese culture.[16] It has been found that although there is only a 17% primary sequence homology between human α-amylase and α-amylase from *A. oryzae*, there is a high degree of structural homology/polypeptide chain folding (70% topological equivalence), especially in the conformations of the active site residues and the site of calcium binding near the active site (virtually identical positioning, binding ligands, and associated water molecules), and that they likely have the same mechanisms of catalytic action.[17] Therefore, this is a suitable model for this device. The α-amylase powder will be mixed in phosphate-buffered saline (PBS) solution as "simulated saliva", since PBS has been used for simulated saliva samples in previous dental research,[18] and since the pH of reaction needs to be tightly regulated. By this principle, enzyme reactivity stops at a pH which is too low, so hydrochloric acid (HCl) will be used as stopping solution for the reaction of α-amylase with potato-derived starch in potassium iodide-iodine solution, for which a general protocol has been developed.[19]

The α-amylase from *Aspergillus oryzae* that will be purchased has a strength of 1.5 activity units/g, in which 1 unit is defined as the mass of α-amylase required to produce 1 μmol of maltose after 1 minute of reaction at a temperature of 25 °C and pH of

6.0. In other words, ~1.95 mg of this specific α-amylase will produce 1 mg of maltose at 25 °C. α-amylase activity ranges for the 4 periodontal classifications (healthy, mild, moderate, or severe) are given in units as well, however, 1 unit is defined as the mass of α-amylase required to produce 1 mg of maltose at 20 °C. Since the absorbance of a sample is proportional to the concentration of starch, the change in absorbance is inversely proportional to the concentration of maltose. It is important to note that the change in absorbance will be measured relative to blanks prepared at the same temperature as the sample, since temperature may affect the iodine color, and that mass can be calculated from concentration, since a constant volume will be used. Therefore, the strength of this specific α-amylase at 20 °C can be can be calculated by preparing the following standard curves (Fig. 1). It is important to note that this preliminary figure assumes that the degradation of starch will occur to a smaller extent at 20 °C compared to 25 °C, and that there will be a higher degree of iodine fixation by starch, leading to a higher baseline absorbance, at 20 °C compared to 25 °C. It is also important to note that available mass scales are not sensitive enough to accurately measure below 10 mg (.01 g), so absorbances at these values will be interpolated using data from relatively higher masses (i.e. the standard curve equations).

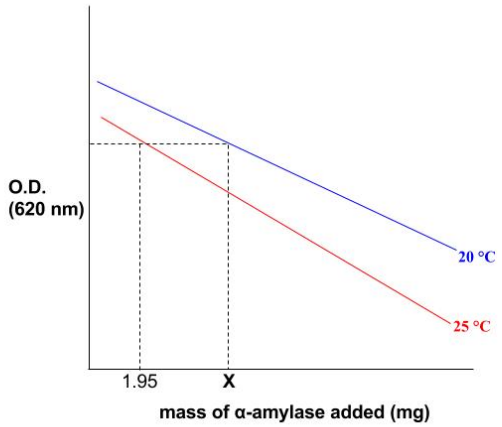

Figure 1: Conversion Protocol (**X** = U/mg of α-amyase)

Upon determining the strength of the specific α-amylase at 20 °C (in units/mg), the x-axis of the above figure can be modified to produce our final figure which relates the change in absorbance to the severity of periodontitis (Fig. 2).

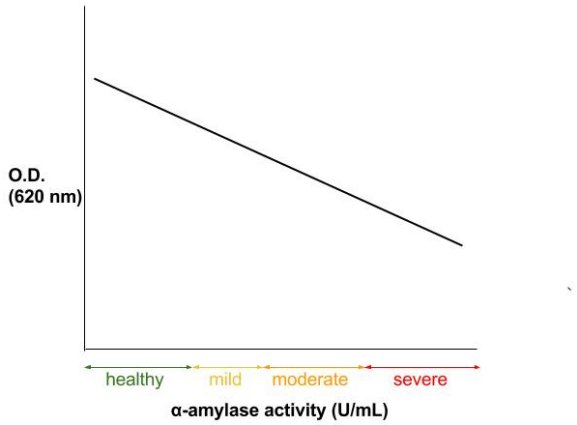

Figure 2: Anticipated Final Standard Curve

The protocol which will be utilized will involve preparing a temperature bath (20 °C or 25 °C), adjusting the pH of PBS buffer to 6.0, dissolving samples of α-amylase whose masses approximately fall into the range of activities relevant to periodontitis (~100-300 mg) in the PBS at the mentioned temperatures, mixing them into homogenized starch solutions (with excess starch) for 1 minute, pipetting small volumes of the resulting solutions to hydrochloric acid "stop solutions", pipetting small volumes of the resulting solutions to potassium iodide-iodine solutions, and pipetting small volumes of the resulting solutions into cuvettes, whose absorbances will be measured with a spectrophotometer, and eventually this device, at 620 nm.

The chemical aspect of this project will be designed to satisfy requirements 4 and 7 in the design matrix (18. b.), which state "Device must require a small sample volume of saliva" and "Device must allow samples collected to be transferred with ease".

A wide variety of other biomarkers were considered, both in the GCF and saliva. These included: MMP-8 (matrix metalloproteinase), IL-1 (interleukin), MFG-E8 (milk fat globule epidermal growth factor 8), OPG (osteoprotegerin), RANKL, (receptor activator for nuclear factor kappa B ligand), LPA (lysophosphatidic acid) LDH (lactate dehydrogenase), ALP (alkaline phosphatase), CRP (C-reactive protein), β-glucuronidase, cortisol, Na^{+}, K^{+}, Ca^{2+}, pH, and temperature. Several were eliminated on the basis of cost alone, in addition to all GCF biomarkers since the sample volume and resulting concentrations would be too small to detect with the resources available. Furthermore, there were not many sources which reported concentrations of some of these biomarkers in the 3 stages of periodontitis, but rather in only healthy, gingivitis, or periodontitis (which would make this device diagnostic and not monitoring of periodontitis). GCF biomarkers would also require the collection of GCF per tooth, which may be time-consuming and not present a significant advantage of this device over the standard probing procedure. Several assay systems were also considered including existing ELISAs (enzyme-linked immunosorbent assays) and fluorescent assays, but they were too expensive. So, this resulted in the selection of a salivary enzyme (α-amylase), for which an inexpensive colorimetric assay protocol already exists and which utilizes inexpensive reagents such as starch and hydrochloric acid. Finally, several orthologs of

α-amylase were considered including human salivary α-amylase, which was too expensive, and bacterial α-amylase from *Bacillus subtilis*, whose enzyme strength was too high, requiring very small masses which available mass scales are not accurate enough to measure. It is important to note that an auxiliary kit containing aliquots of additional reagents (PBS, starch, HCl, and potassium iodide-iodine solution) whose volumes correspond to the amounts needed to analyze one saliva sample will be provided with this device (along with test tubes and pipettes).

Requirement 4 will be verified by running the device with 0.82 mL of simulated saliva three times, to confirm that this is the minimum volume needed. Requirement 7 will be verified by timing the pre-mixing and placement of cuvettes of known volumes into the device five times, and measuring the volume afterwards, to confirm that pre-mixing takes less than 2 minutes $\pm$ 30 seconds (not including the actual reaction time) and that no sample volume is lost during their placements into the device.

The device will be validated by testing the device with simulated saliva with 5 α-amylase activities (whose concentrations will be known) per periodontal severity, and comparing them with the LCD outputs (severities and concentrations). An error of $\pm$ 10% is acceptable.

Chapter 3: Electrical Aspect (Sindhuja Kuchibhatla)

The electrical component of the Periodontal Monitoring Device begins with the LED that transmits the incident light, which will be used as to measure the absorbance of the saliva sample solution. The LED that we will be using for our design is a super bright (1500 mcd) red LED with a 620 nm wavelength and a diameter of 5mm. It is essential to read the absorbance of the salivary amylase at 620 nm because the structure of alpha-amylase can absorb light strongly at that specific wavelength. The forward voltage of the LED is 1.8-2.2V so it is acceptable to use a 9V battery to power this LED. However, a resistor is needed to ground the remaining voltage (6.8-7.2V) coming out of the battery to make the LED work.

The second electrical element is the light sensor, which will be placed on the other side of the cuvette to measure the amount of light that is transmitted through the solution. For this, photo-resistors were considered, whose resistance decreases with increasing incident light intensity, and photodiodes, which convert light into current that can be measured. However, neither of these options would produce as precise of an output as the Adafruit TSL2561 Luminosity Sensor. The TSL2561 is an advanced digital light sensor that has a wide range for detecting and measuring light (0.1-40,000 Lux). This sensor has low power consumption due to its low current draw of only 0.5 mA when actively sensing and 15 μA when in power-down mode. The TSL2561 gives the user the advantage of configuring the gain and integration time setting, which optimize the sensing for each specific condition that it is used in. Shorter integration times will allow less light into the device when it is used in bright conditions, whereas longer integration times will allow more light into the device for dim lit conditions. The light sensor has a default gain setting of X1, which is better used for bright conditions, but can go up to a setting of X16, which has a sensitivity that is sixteen times greater than that of the default and is used for dim conditions. These two settings will be changed according to the light conditions of the casing of our Periodontal Monitoring Device.

The Lux data obtained from the TSL2561 light sensor is then processed by a microcontroller. Since the TSL2561 light sensor has a built in ADC, it can be used with any microcontroller but for this device, the Arduino UNO R3 was chosen. Arduino products are extremely accessible and highly flexible to be customized according to the device needs. This type of microcontroller is also inexpensive, costing $24.95 per board and comes with a whole selection of codes that have been previously made and posted in the Arduino libraries online. The Arduino will be used to convert the Lux data into a concentration of the α-amylase after calculating the standard curve for absorbance versus concentration to establish the relationship of the two. Before conducting experiments using the device, the light sensor will be calibrated using a “blank” measurement at least once a day to ensure accurate readings. The “blank” measurement simply involves carrying out the device function using a clear solution, such as distilled water.

To wire up the TSL2561 to the Arduino, a soldering method to include a five-pin length of a male-male header strip will be used. Jumper wires (male/female) will be attached to this header strip to establish a connection between the Arduino and the TSL2561 sensor. Although the TSL2561 sensor comprises of 6 pins (GND, VIN, ADDR, INT, SDA, and SCL), only four of the six pins (GND, VIN, SCL, and SDA) will be used to connect to the Arduino. The remaining pins are not needed for the function of this device. The VIN and GND pins will be used to provide a 2.7-3.6V supply. It is essential

to remain in this range of voltage power supply because higher voltages will permanently damage the light sensor and lower voltages will not provide adequate power to make it work. Therefore, the VIN pin will be connected to the 3.3V pin on the Arduino to ensure that the power is within this range and the GND pin will be connected to the GND pin of the Arduino. The SDA pin will be hooked up to the pin labeled A4 on the Arduino Uno and the SCL pin will be connected to the A5 pin on the Arduino. After these connections are established, the Arduino will be able to categorize the concentration of the biomarker into levels of mild, moderate, or severe according to the intervals shown by Table 1 below. Finally, an LCD display will be used to display the level of the periodontitis to the user. The LCD chosen for this device is the Adafruit Standard LCD 16x2 + extras, which has white text on a blue background.

As previously mentioned, a variety of different light sensors were considered to measure the absorbance or light transmitted through the solution. These include photo-resistors and photodiodes, which gave an output of either resistance or current, respectively, as a function of the amount of light absorbed. Both of these light sensors were very inexpensive, however their sensitivity and precision were not as good when compared to the Adafruit TSL2561 light sensor.

The microcontroller (Arduino) component will aim to satisfy requirement 2 of the Design Matrix (Appendix B), which states, "Device must classify concentration of biomarker as a disease severity level". The specification for this requirement states, "Device must be able to match measured values of α-amylase to established ranges that define each periodontal severity[13]". This requirement is warranted for the function of this device because levels of α-amylase increase in relation to the progression of periodontal disease[13]. Again, these established ranges are shown in Table 1 below. In order to accommodate saliva samples that will have borderline measurements, marginal levels of severity (Mild-Moderate, Moderate-Severe, etc.) will be considered.

Healthy	**Mild**	**Moderate**	**Severe**
< 86.31 U/mL	86.31-118.8 U/mL	118.8-139.5 U/mL	> 139.5 U/mL

Table 1: α-amylase levels in various severity levels of periodontitis.

The verification plan for this specification is to make ten solutions of varying known concentrations of biomarker and test the device to confirm that the device will accurately classify the concentration as a disease severity level. The validation protocol includes making simulated saliva samples (5 of each severity level) and running through the entire process of the device. Final results from the LCD screen and the known concentrations entered will be compared.

Chapter 4: Mechanical Aspect (Lindsey Cabanas)

To measure the proper absorbance and correlate it to the concentration of amylase, a casing is necessary for the device. It is imperative that the casing does not allow interference from any outside light sources. Interference could change the light absorbance and could cause misreadings from the light sensor. This misreading would result in an inaccurate final output to the user. For selecting the material for the black box, the function and the budget were the main considerations. The design for the "blackbox" can be seen below, in Appendix F. It will incorporate a rectangular shape for the casing.

The casing will be made of black "chemical-and wear-resistance acetal", with a thickness of 1/8th of an inch. This material is chemical resistant to chemicals such as solvents and alcohols, which would possibly be used in the cleaning of this device. This material of acetal, which is a plastic, is also able to withstanding some stretching, as its tensile strength is 6,400-9,500 psi. This strength is enough for the purposes of the device as it will not be moved around or jostled. This material can be machined with high-speed steel tooling. The connection of the different sides of the "blackbox" will be attached using a glue, cyanoacrylate adhesive. The material will have to be cleaned first, using a primer, to ensure the best adhesive aspect. A hinge will be used to make the top open and close effectively to put samples inside of the device. The "blackbox" will also incorporate the LED light used into the side of the device. The LED will be held inside the wall of the box using a LED holder, which will help to ensure that no movement of the light will occur.

It is imperative that while measuring the absorbance, the distance of cuvette from the light source remains the same for each trial. It is also important that the distance of the cuvette to the Adafruit board to remain the same. For this reason the design will incorporate a cuvette holder, so that the user of the device will be able to place the cuvette in the same measured location every time. The cuvette holder will also be used to make sure that the light is hitting the center of the cuvette during usage. Because of this, the cuvette holder might have to be raised in order to keep the sample at a height that will directly be in the light of the LED. This will be crucial to getting an accurate reading of light absorbance. It is the hope that the cuvette holder will be made of the same material as the black box. Figure 3 below shows the integration of the black box and the cuvette holder with the electrical components of the device, like the battery, light sensor and the hole where the LED and LED holder would be located. In addition to the black box and the cuvette holder, a platform will be incorporated to the design, as seen in Figure 3. This will raise the device off of any surface that it is placed on.

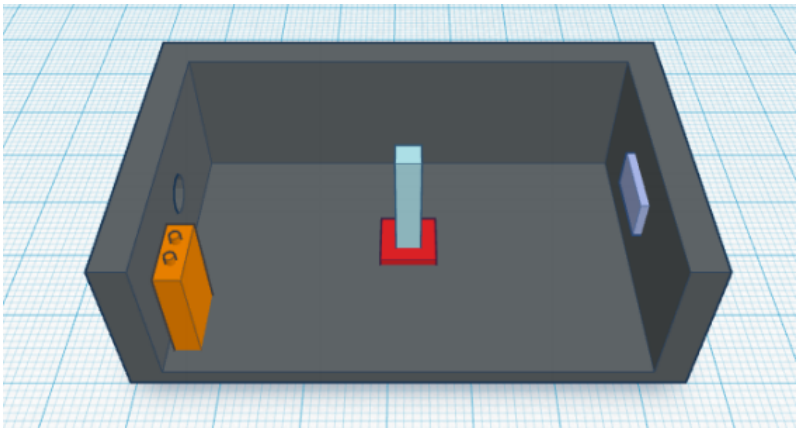

Figure 3: Integration of the black box and the cuvette with the electrical components of the device.

The requirements that pertain to the mechanical aspect of the design solution are requirement numbers 5 through 8. Requirement 5 states that the "Device must not be susceptible to corrosion or degradation caused by the reagents used". The specification goes on to state that the "device walls must be made of chemically inert materials" and also that "reagents housed inside the device must not cause any degradation or corrosion for one week". The justification is that the device should not experience corrosion or degradation due to the chemicals that may contact the surface during use. Requirement 6 states that the device sample chamber and samples used for testing must be able to be safely discarded. The specification is that no additional protective gear must be needed to handle the materials. This shows that the device should not be detrimental to the user. Also to help meet this requirement we will be using disposable cuvettes in which they can be discarded after testing has occurred. For device requirement 7, the device must allow the transfer of samples into the device with ease. The specification includes no loss of specimen, and also that insertion should take no more than two minutes, with a standard error of thirty seconds. This is due to the fact that this device is supposed to be a helpful device to the dentist and dental professionals. If this device shows a difficulty to use or place samples inside, it will be more of a hindrance to the user. For device requirement 8 states that the device must be able to resist moderate cleanings between each patient. The specification states that it must be able to withstand the concentrations of a standard bleach solution. This is due to the fact that these samples might contaminate the device, and it would be helpful and add to the ease of use if it could be cleaned between uses.

While making decisions about which type of material to build the black box, many different materials were considered. Black hardboard and black poster-board were both considered for construction. These materials were not only extremely expensive and would be a major cost to the budget, but they would not be able to withstand the cleaning of the black box, as stated in the design requirements.

For requirement 6, the verification plans include housing the reagents in the separate components of the casing for one week. This time period was chosen because typically the test will take significantly less time than one week, approximately 10 minutes or less, but this time period allows for adequate time to see if any leakage will occur. For requirement number 7 there are no verification activities planned. For

requirement 8, the verification plans include an insertion of the cuvette into the device. This insertion will be timed, and also the volume will be measured before and after insertion. For requirement 9, the devices will be cleaned using the standard bleach solution ten times. Throughout these cleanings, the device will be monitored for change in shape or function. For requirements 6-9, there are no validation plans.

Chapter 5: Budget & Schedule

5.1 Cost Estimate/Budget

As of the current materials list (Appendix K), the total cost is $285.30. The list includes the chemical components of this design, α-amylase from *A. oryzae* to create the standard curves on which this device will be based and for verification/validation, potato-derived starch substrate, potassium iodide-iodine colorimetric indicator for the reaction, hydrochloric acid to stop the reaction, phosphate buffered saline to regulate the pH and prepare simulated saliva solutions, the electrical components of this design, which include the Arduino UNO microcontroller, alkaline 9V battery to power the device, LED to produce incident light, Adafruit TSL2561 light sensor to detect transmitted light, LCD display to present severity, LED holder to prevent dislocations of the LED, and the mechanical components of this design, which include primer for cleaning, a cyanoacrylate adhesive glue, a chemical/wear-resistant acetal sheet for the casing, an unfinished steel surface, and light-absorbing black-out paper to minimize interference from other sources of light.

5.2 Schedule

According to the Gantt Chart (Appendix J), in January, chemical reagent volumes will be optimized so that the standard curves are linear while the change in absorbance is significant, arduino codes will be written to measure the absorbance and output both concentration (U/mL) and severity, and device dimensions will be finalized. In February, standard curves for the reaction at both temperatures will be created, all electrical schematics will be created, the mechanical parts of the device will begin to be machined, and an abstract to the Northeastern Bioengineering Conference (NEBEC) with anticipated results will be submitted. In March, stage 1 of validation will be initiated, in which simulated saliva in the healthy and mild stages of periodontitis will be prepared, Requirements (Appendix B) 4 and 7 (chemical), 1 and 2 (electrical), and 5 and 8 (mechanical) will be verified, the electrical components into the device will begin to be affixed, the machining of the mechanical parts of the device will be completely finished, and the interim presentation before spring break will be prepared for. In April, stage 2 of validation will be initiated, in which simulated saliva in the in the moderate and severe stages of periodontitis will be prepared, and NEBEC will be attended. Finally, in May, the Senior Project II Final Presentation and Final Paper will be prepared for.

Chapter 6: Conclusion

Simulated saliva samples will be used for both verification and validation. The saliva sample tests will include different types of tests that will ensure that the classification of the sample including mild, moderate, or severe periodontitis is accurate. The anticipated results of this project's testing are that the device will be fully functioning and giving results after testing in the spring semester.

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Appendix A: Team Member Biography

Sindhuja Kuchibhatla

Sindhuja Kuchibhatla is a senior biomedical engineering student with an electrical engineering concentration at The College of New Jersey from Kendall Park, NJ. Upon graduation, she hopes to attend graduate school to pursue a Master's in Biomedical Engineering. She would eventually like to work in the biomedical engineering industry, ideally focusing on neural or clinical engineering. Outside of engineering, she is Treasurer of the Indian Student Association and a member of the TCNJ Jiva dance team.

Naina Iyengar

Naina Iyengar is a senior biomedical engineering-electrical concentration major at the The College of New Jersey from South Brunswick, NJ. After college, she plans on attending dental school. She is also currently the Vice-President of TCNJ's Pre-Dental Association and is a representative on the Student Advisory Board.

Lindsey Cabanas

Lindsey Cabanas is a senior biomedical engineering - mechanical concentration major at The College of New Jersey from Somerset, NJ. After graduating from TCNJ, she plans on attending graduate school to earn her Master of Business Administration. Apart from academics, Lindsey is a sister of Sigma Sigma Sigma, and chair of Alumni Relations.

Appendix B: Design Matrix

Design Input		Verification Activity	Validation Activity
Device Requirements	**Device Specifications**	**Method/Protocol**	**Method/Protocol**
1. Device must measure a salivary biomarker that relates to periodontal disease progression[1]	1.1 Device must measure α-amylase activity in the saliva in a range from 86.31 U/L to 139.5 U/L [1]	Ten solutions of varying known concentrations of biomarker will be made and tested within the device to confirm that the device accurately correlates the concentration to the severity level.	Simulated saliva samples (5 of each severity level) will be made. Samples will be entered into the device and run through the entire process. Final results from the LCD screen, the standard curve, and the known concentrations entered will be compared.
2. Device must classify concentration of biomarker to disease severity[2]	2.1 Device must be able to match measured values to established ranges that define each periodontal severity (mild: 86.31-118.8 U/L, moderate: 118.8-139.5 U/L, severe: > 139.5 U/L) [1]		
3. Device must present the severity level of the disease to the user	3.1 Device must use an LCD display to relay the severity level (mild, moderate, or severe)		
4. Device must require a small sample volume of saliva[3]	4.1 Device must be able to measure the selected biomarker with a minimum of 0.82 mL of saliva	The device will be utilized using a sample of only 0.82 mL and run to completion. This will occur for three samples of this size, to confirm that a minimum sample size of 0.82 mL is needed to complete test.	

Justifications:

[1] α-amylase increases in relation to the progression of periodontal disease [1]

[2] Ranges of salivary α-amylase concentrations for the different severity levels have been found by previous studies [1]

[3] Current commercial oral fluid devices collect an average of 0.82-1.86 mL of saliva [2]

Design Input		Verification Activity	Validation Activity
Device Requirements	**Device Specifications**	**Method/Protocol**	**Method/Protocol**
5. Device must not be susceptible to corrosion or degradation caused by the reagents used[4]	5.1 Device walls must be made of chemically inert materials 5.2 Reagents housed inside the device must not cause any degradation or corrosion for one week	The reagents will be housed in their separate mechanical components for one week. Throughout the week and after the week, the components will be checked for degradation and wear.	
6. Device sample chamber and samples used for testing must be able to be safely discarded[5]	6.1 No additional protective gear should be necessary to handle the chemicals apart from regular dentist gloves		
7. Device must allow samples collected to be transferred with ease[6]	7.1 There should be no loss of specimen volume while inputting the sample into the device 7.2 Inputting the sample into the machine should take no more than 2 minutes ± 30 seconds	Cuvettes with known volumes of liquid will be placed into the device five times. These trials will be timed. The volumes will also be measured before and after to confirm that the sample size has not decreased in the transfer of the sample.	

[4] Incompatible materials for iodine include water-reactive materials, metals (ferrous), rubber, and plastics [3]
[5] Guidelines are set by the EPA and FDA on how to properly dispose of chemicals that could be corrosive or considered bio-hazardous waste [4]
[6] Ease of use of this device is dependent upon putting the sample into the device without it being a time-consuming activity

Design Input		Verification Activity	Validation Activity
Device Requirements	**Device Specifications**	**Method/Protocol**	**Method/Protocol**
8. Device parts/containers that come into contact with saliva must be resistant to moderate cleaning without impacting function of the device before use for each new patient[7]	8.1 Device parts must be able to withstand cleaning from solutions with 5.25%-6.15% sodium hypochlorite (corrosive cleaning agent in bleach) depending on the manufacturer	The device will be cleaned using standard bleach solution ten times. During and after these cleanings the devices will be observed for change in shape and function.	
9. Materials for the device must cost less than the budget for the device allotted by the engineering department[8]	9.1 All materials for the device must cost below $300 + possible additional funds		
10. Device must be manufactured with existing equipment and facilities[9]	10.1 Existing equipment and facilities are those found in Armstrong Hall or the Science Complex of TCNJ		

[7] Transmission of periodontal pathogens (Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis) can occur in adult individuals [5]

[8] Each team is granted $100 per member, giving us our team a total of $300 + any additional funds that may be granted

[9] Using external sources for manufacturing will be expensive, risky, and difficult to find

References for Design Matrix:

[1] Sanchez, G.A., Miozza, V.A., Delgado, A., and Busch, L. Relationship between salivary mucin or amylase and the periodontal status. Oral Diseases. 19(6). 585-591. 2013.

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[4] Hazardous Waste Regulations. United States Environmental Protection Agency. http://www.epa.gov/wastes/laws-regs/regs-haz.htm

[5] Van Winkelhoff, A. J., and Boutaga, K. Transmission of periodontal bacteria and models of infection. Journal of Clinical Periodontology. 32(6). 16-27. 2005.

Appendix C: Realistic Constraints

Health & Safety

Design must be safe in addition to cost effective because it will be used by medical professionals and will also affect the patient. This constraint applies to the requirements 6 and 9, which state that the "Device must not be susceptible to corrosion or degradation caused by the reagents used" and "Device must not be susceptible to corrosion or degradation caused by the reagents used," respectively.

Environmental

Disposable components may be a biohazard because they are in contact with body fluids such as saliva, GCF, or blood. This constraint applies to the requirement 7, which will ensure that the user is aware of the possible special needs for disposing of the sample. The chemicals involved in manipulating the sample to achieve results are also in need of possible special disposal. This should be able to be communicated effectively to the potential user so that it is handled properly.

Economic

The dean only provides $100 per team member. This constraint applies to the requirement 10, which states that "Materials for the device must cost less than the budget for the device allotted by the engineering department."

Appendix D: Engineering Standards, Specifications, and Codes

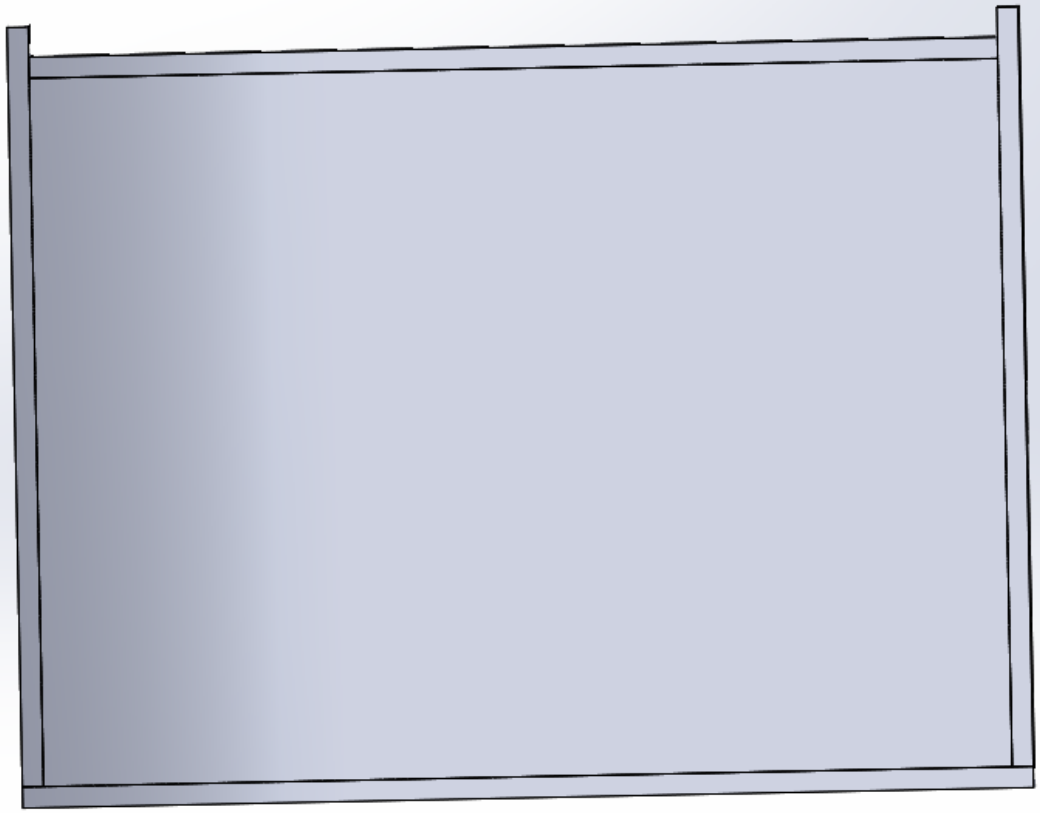
The Occupational Safety and Health Administration (OSHA) has several health and safety codes that are relevant to our project, the first being 1910.138: Hand Protection, which specifies that we wear gloves when handling potentially harmful chemical substances. The second is 1910.303: Electrical-General, which states that electric equipment shall be free from recognized hazards that are likely to cause death or serious physical harm to employee. Finally, the third is 1910.1030: Bloodborne Pathogens, which considers the safety of employees who experience occupational exposure, meaning reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties.

Appendix E: Tools Employed

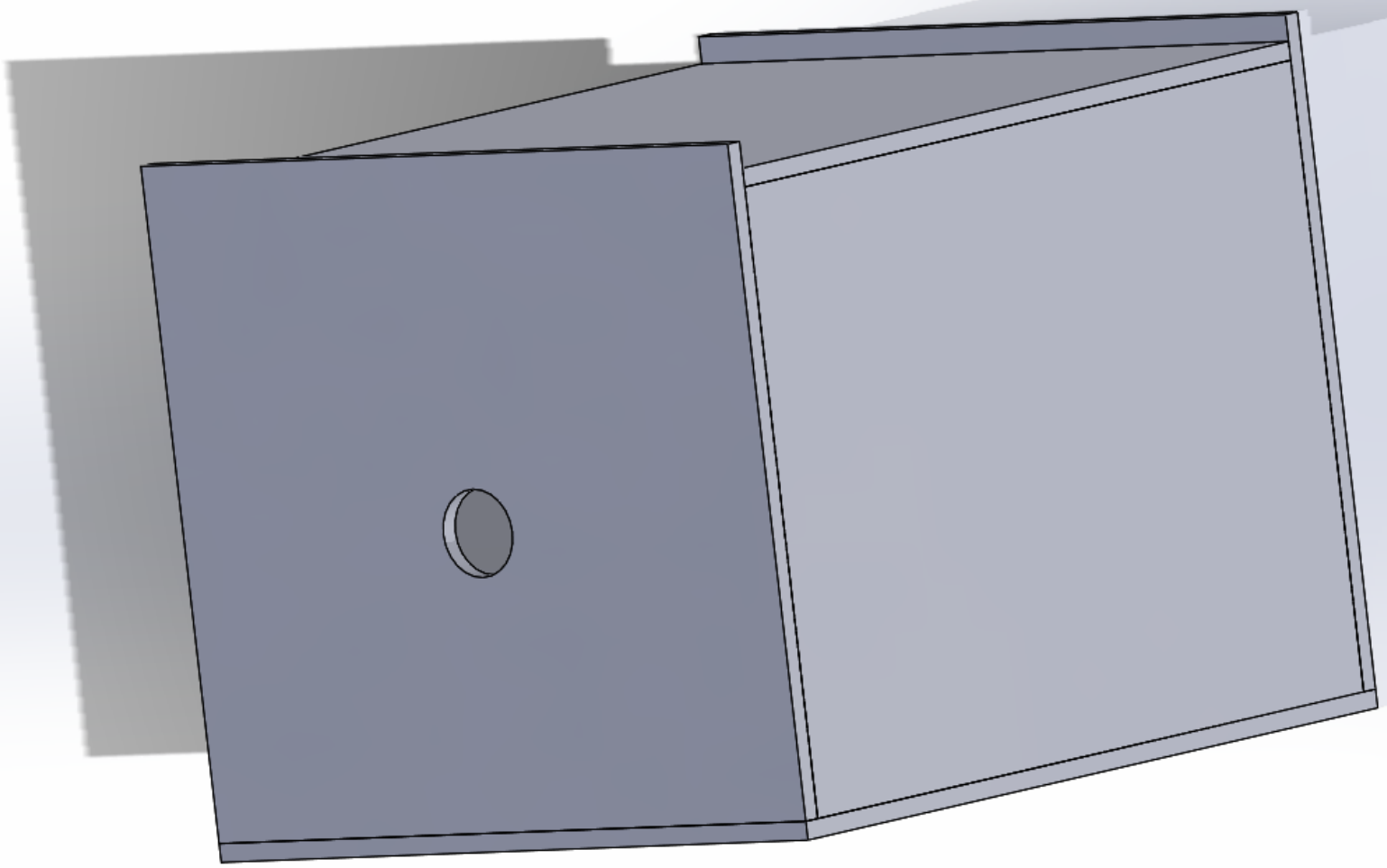
For the development of this device, we have used SolidWorks for the drawings of the casing and the individual component holders. We will be using Arduino libraries in the future to develop codes for the Arduino Uno. We will also be using the equipment available in the TCNJ Machine Shop to produce a prototype of this device and the spectrophotometers available in the TCNJ Science Complex to verify our device.

Appendix F: Drawings

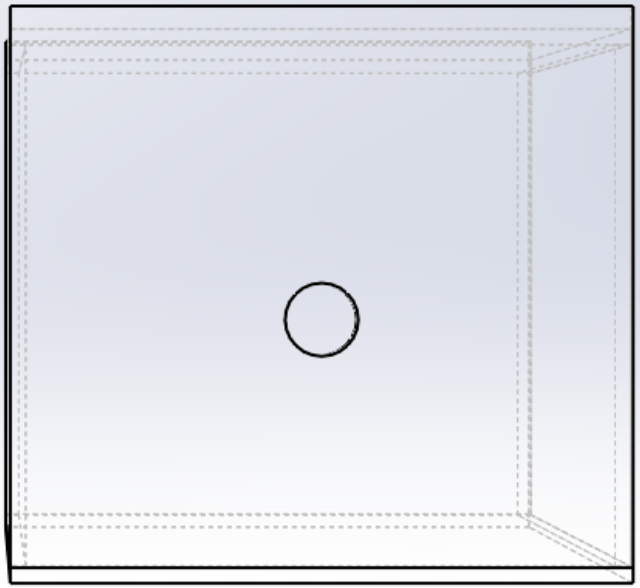
Black Box Assembly Rear View

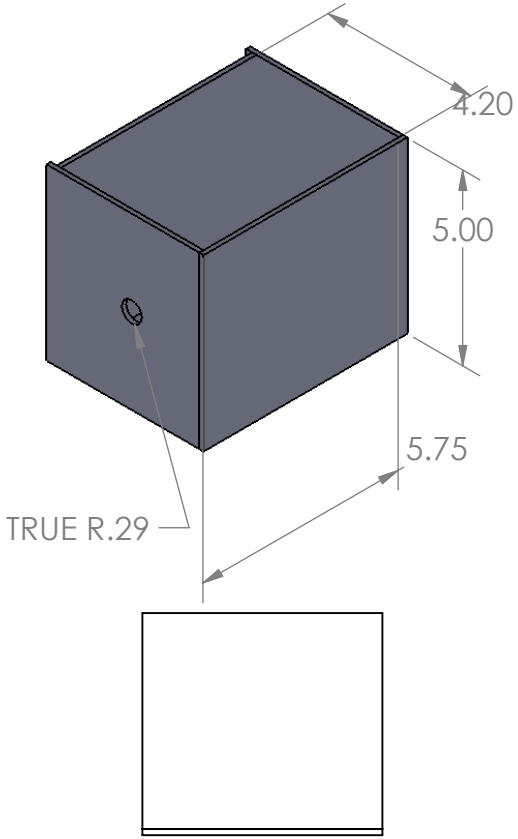


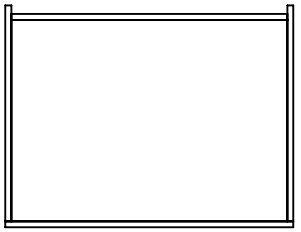
Black Box Assembly Full View

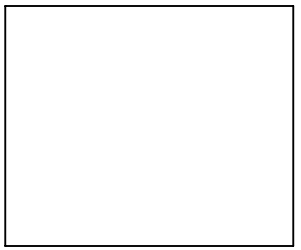


Black Box Assembly Front View









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		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±	DRAWN		
			CHECKED		
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			MFG APPR.		
		INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.		
		MATERIAL	COMMENTS:		
NEXT ASSY	USED ON	FINISH			
APPLICATION		DO NOT SCALE DRAWING			

PROPRIETARY AND CONFIDENTIAL

THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF <INSERT COMPANY NAME HERE>. ANY REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF <INSERT COMPANY NAME HERE> IS PROHIBITED.

TITLE:

Black Box Assembly for Periodontal Monitoring Device

SIZE	DWG. NO.	REV
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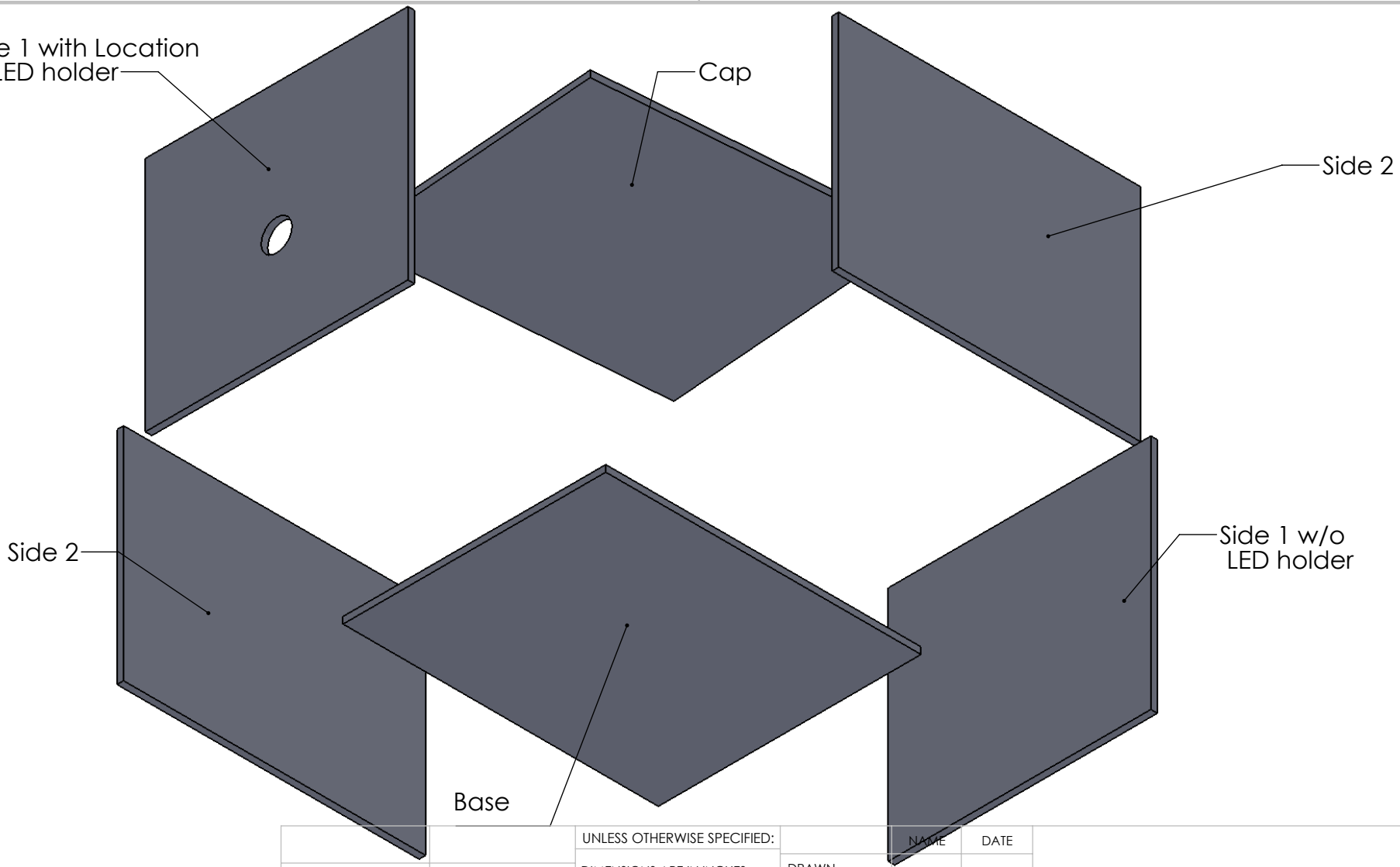
SCALE: 1:4	WEIGHT:	SHEET 1 OF 1



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		MATERIAL	COMMENTS:		
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APPLICATION		DO NOT SCALE DRAWING			

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TITLE:

Black Box Expanded View

SIZE	DWG. NO.	REV
A	drawingexpandedview	
SCALE: 1:8	WEIGHT:	SHEET 1 OF 1

Appendix G: Computer & Microcontroller Codes

Computer & Microcontroller codes will be added in the near future.

Appendix H: Ethical Considerations

Since fungal α-amylase will be used to design this device and for preparation of simulated saliva during verification/validation, there are no ethical considerations related to human or animal testing which will be involved, but in the future (Senior Project II Final Report), an IRB proposal will be filled out as if saliva samples from human participants will be tested with.

Appendix I: Meeting Minutes

9/9/2015
Members met with Dr. Hall and discussed the upcoming presentation. The slides must be completed and sent to Dr. Hall by Sunday morning. On our Monday morning meeting we will run through the entire presentation. Show what we plan to detect and why, is the device going to be an external device or an internal device, who is the user, what kind of information does it give to the dentist, and how will it be used. Inside the introduction of the device do not forget to include the methodologies of other devices and background information on the disease.

9/21/2015
Members met to discuss the different biomarker detection and to also divide up the different roles of the project itself. The problems and goals that need to be addressed in the future are to determine the exact biomarker that will be detected, determine how the biomarker correlates to the disease, and determine how the reagents are going to be delivered for this process.

9/27/2015
This week the team members had their initial meeting with Mr. Joe Zanetti. The team members also narrowed down their choices of biomarkers to alpha-amylase, LDH, and pH. All of the possible biomarkers discussed needed optical sensors. In the next week, the biomarkers will be further narrowed. The team will continue with the design process, and also consider the different types of optical sensors to possibly use.

10/5/2015
Prior to this meeting the team members were able to correlate alpha-amylase and LDH to periodontal disease activity found. The optical sensor discussed was a colorimetric sensor. For the future goals and tasks this week, the sensitivity of the colorimetric sensor must be further researched. Also the biomarkers must be narrowed down to one. The casing for the device must be considered and worked on.

10/14/2015
Discussed that alpha-amylase has been chosen. The team also discussed the differences between a photodiode and a light emitting diode. The discussion of how much purified amylase will fall within the range, and also discussed the need to get the MSDS forms for all of the chemicals being used in the reaction. The requirements from the presentation must also be changed to incorporate the feedback from the presentation.

10/19/2015
Prior to this meeting the team members chose alpha-amylase as a biomarker. Different methods of light sensors were investigated that could potentially be used for colorimetric detection, such as a photo-resistor and a photodiode. The kinetics of the alpha-amylase reaction that would produce color was also investigated. Initial steps of designing the casing were considered as well. In the future, the team needs to specify which light sensor to use and examine the advantages and disadvantages of each. For future

considerations, how the absorbance will be converted into the concentration of the biomarker will also need to be considered.

10/27/2015
During this meeting the use of a microcontroller to correlate the absorbance measurement to the biomarker concentration. Currently, the photo-resistor will be used to sense the changes in the light. For the next meeting, verification and validation activities must be discussed for each device requirement.
11/2/2015
The different types of material for the casing were considered. The micro-controller selected was the Arduino Uno. The materials for the LED holder, cuvette holder, and light sensor holder are being considered. Also discussed was the protocol for converting absorbance to severity level. There are two different types of alpha-amylase that could possibly be used for testing. For the future, the drawings need to be started for the holders and casing. The sensitivity for available mass scales is needed for verification and validation. The final list of reagents needs to be compiled.

11/9/2015
The safety survey for the machine shop has been completed. The selection of the alpha amylase sample has also been completed. The light sensor has been changed from a photo resistor to a TSL2561 sensor. There has been discussion with Joe about materials for the casing and holders. The holders are being considered for 3D printing for manufacturing. The tasks to be completed are the ordering of electronic devices and chemical reagents. Also, to work on the drawings for the casing and the holders.

11/11/2015
The future tasks that were discussed are the coding of the arduino board and the dimensions on all of the electrical components. In addition to the machined parts requests turned into Joe. Also to determine the sensitivity of the mass scales and determine any other reagents needed for the completion of the reaction.

11/16/2015
During this meeting, a draft of the abstract was completed and turned into Dr. Hall for review. For the next meeting, the changes should be incorporated into the abstract. At the meeting the changing of certain requirements for the design matrix was discussed. For next meeting, research the importance of the height of the liquid in the cuvette, or the need for a certain volume as it pertains to the sample.

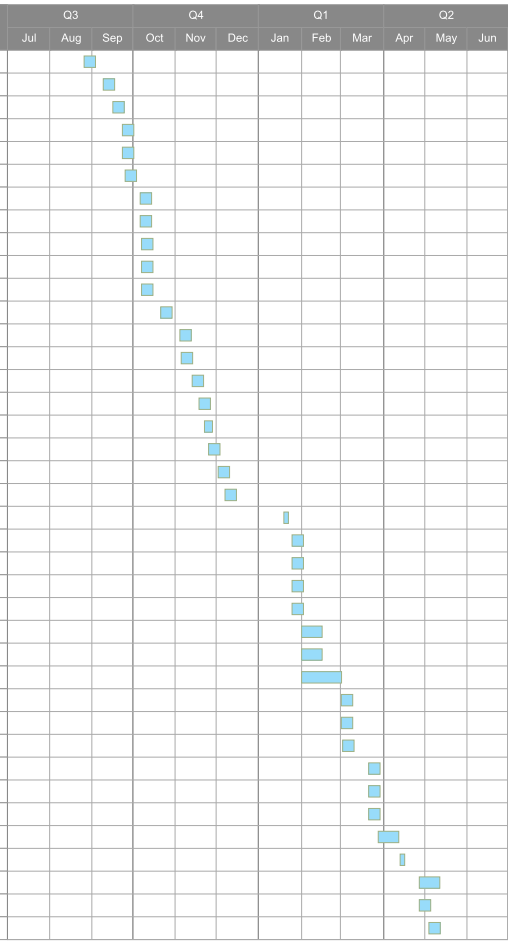
11/23/2015
During this meeting we discussed how to gain access to the use of the chemistry laboratory with the spectrophotometer. Also discussed the differences between the bacterial and fungal alpha-amylase to use for possible testing.

11/30/2015
During this meeting, changes for the final fall presentation were discussed. Naina's future task includes finding if saliva volume changes needed starch concentration. Future tasks for the entire team include incorporating the "kit aspect" of the design.

Appendix J: Gantt Chart

Senior Project

	Task Name	Duration	Start	Finish	Owner
1	Device Requirements	6d	08/26/15	09/02/15	All
2	Device Requirements Presentation	6d	09/09/15	09/16/15	All
3	Web Site	6d	09/16/15	09/23/15	Sindhuja
4	Realistic Constraints	6d	09/23/15	09/30/15	All
5	Standards	6d	09/23/15	09/30/15	All
6	Machine Shop Meeting	6d	09/25/15	10/02/15	Lindsey
7	Chose Biomarker	6d	10/06/15	10/13/15	Sindhuja
8	Chose Assay System	6d	10/06/15	10/13/15	Sindhuja
9	Submit Travel Requests	6d	10/07/15	10/14/15	Naina
10	Budget	6d	10/07/15	10/14/15	Naina
11	Gantt Chart	6d	10/07/15	10/14/15	Naina
12	Interim Design Review Presentation	6d	10/21/15	10/28/15	All
13	Complete Drawings	6d	11/04/15	11/11/15	Lindsey
14	Safety Survey	6d	11/05/15	11/12/15	Naina
15	Abstract	6d	11/13/15	11/20/15	Sindhuja
16	Final Report-Draft	6d	11/18/15	11/25/15	All
17	Order Electrical Materials	6d	11/22/15	11/27/15	Sindhuja
18	SP I Final Presentation	6d	11/25/15	12/02/15	All
19	SP I Final Report	6d	12/02/15	12/09/15	All
20	Order Chemical Materials	6d	12/07/15	12/14/15	Naina
21	Optimize Reagent Volumes	3d	01/19/16	01/21/16	Naina
22	Write Arduino Codes	6d	01/25/16	02/01/16	Sindhuja
23	Finalize Device Dimensions	6d	01/25/16	02/01/16	Lindsey
24	Order Mechanical Materials	6d	01/25/16	02/01/16	Lindsey
25	Create Standard Curves - 20 C and 25 C	6d	01/25/16	02/01/16	Naina
26	Northeastern Bioengineering Conference Abstract	11d	02/01/16	02/15/16	All
27	Electrical Schematics	11d	02/01/16	02/15/16	Sindhuja
28	Begin Machining	21d	02/01/16	02/29/16	Lindsey
29	Finish Machining	6d	03/01/16	03/08/16	Lindsey
30	Affix Electrical Components	6d	03/01/16	03/08/16	Sindhuja
31	Interim Presentation	6d	03/02/16	03/09/16	All
32	Verification: Requirements 4 & 7	6d	03/21/16	03/28/16	Naina
33	Verification: Requirements 1 & 2	6d	03/21/16	03/28/16	Sindhuja
34	Verification: Requirements 5 & 8	6d	03/21/16	03/28/16	Lindsey
35	Validation	11d	03/28/16	04/11/16	All
36	Attend Northeastern Bioengineering Conference	3d	04/13/16	04/15/16	All
37	Final Report - Draft	11d	04/27/16	05/11/16	All
38	SP II Final Presentation	6d	04/27/16	05/04/16	All
39	SP II Final Report	6d	05/04/16	05/11/16	All



Appendix K: Material List

1. a-amylase from *Aspergillus oryzae*, powder, ~1.5 U/mg
2. Phosphate buffered saline, powder, pH 7.4
3. Starch, Soluble Potato, ACS Grade, 100 g
4. Iodine-Potassium Iodide Solution, Laboratory Grade, 500 mL
5. Hydrochloric Acid, 0.1 M (0.3% v/v), Reagent Grade, 1 L
6. Adafruit TSL2561, Digital Luminosity/Lux/Light Sensor Breakout
7. Arduino Uno R3 (Atmega328-assembled)
8. Standard LCD 16x2 + extras – white on blue
9. Super Bright Red 5mm LED (25pack)
10. 5mm Plastic Flat LED Holder – Pack of 5
11. Alkaline 9V Battery
12. Loctite 770 Primer 1.75-oz. Bottle Semi-Clear
13. Loctite Prism 454 Cyanoacrylate Adhesive
14. Chemical-and-Wear Resistant Acetal, Sheet, 1/8" Thick, 12" x 24", Black
15. Unfinished Steel Surface-Mount Hinge without Holes, Nonremovable Pin, 1" High, 1" Wide, .047" Thick (2)
16. Light-Absorbing Black-Out Paper, Plain Back, 27" x 36", 0.01" Thick

Appendix L: Financial Budget

Budget

Item	Company	Quantity	Part #/Stock #	Unit Cost ($	Shipping Cost ($)	Hazards	Total Cost ($	Pur	Exp	Act	Co	Notes	
a-amylase from Apergillus oryzae, powder, ~1.5 U/	Sigma-Aldrich Co.,	1	86250-100G	48.20	12.39	respiratory sensitation	60.59	TBD	TBD	TBD	http	N/A	
Phosphate buffered saline, powder, pH 7.4	Sigma-Aldrich Co.,	3	P3813-1PAK	5.70	included above ^	nonhazardous	17.10	TBD	TBD	TBD	http	1 packet in 100 mL is 0.1 M	
Starch, Soluble Potato, ACS Grade, 100 g	Carolina Biological	1	892529	16.75	11.18	nonhazardous	27.93	TBD	TBD	TBD	http	N/A	
Iodine-Potassium Iodide Solution, Laboratory Grad	Carolina Biological	1	869055	9.40	included above ^	harmful if swallowed/t	9.40	TBD	TBD	TBD	http	N/A	
Hydrochloric Acid, 0.1 M (0.3% v/v), Reagent Grad	Carolina Biological	1	867823	8.30	included above ^	causes skin and eye ir	8.30	TBD	TBD	TBD	http	N/A	
Adafruit TSL2561, Digital Luminosity/Lux/Light Sen	Adafruit Industries	1	439	5.95	7.01	N/A	12.96	TBD	TBD	TBD	http	N/A	
Arduino Uno R3 (Atmega328-assembled)	Adafruit Industries	1	50	24.95	included above ^	N/A	24.95	TBD	TBD	TBD	http	N/A	
Standard LCD 16x2 + extras – white on blue	Adafruit Industries	1	181	9.95	included above ^	N/A	9.95	TBD	TBD	TBD	http	N/A	
Super Bright Red 5mm LED (25pack)	Adafruit Industries	1	297	8.00	included above ^	N/A	8.00	TBD	TBD	TBD	http	N/A	
5mm Plastic Flat LED Holder – Pack of 5	Adafruit Industries	1	2175	0.95	included above ^	N/A	0.95	TBD	TBD	TBD	http	N/A	
Alkaline 9V Battery	Adafruit Industries	1	1321	2.50	included above ^	N/A	2.50	TBD	TBD	TBD	http	N/A	
Loctite 770 Primer 1.75-oz. Bottle Semi-Clear	TBD	1	66205A24	16.78	TBD	N/A	16.78	TBD	TBD	TBD	TB	N/A	
Loctite Prism 454 Cyanoacrylate Adhesive	TBD	1	74765A35	25.79	TBD	N/A	25.79	TBD	TBD	TBD	TB	N/A	
Chemical-and-Wear Resistant Acetal, Sheet, 1/8" T	TBD	1	TBD	22.96	TBD	N/A	22.96	TBD	TBD	TBD	TB	N/A	
Unfinished Steel Surface-Mount Hinge without Hole	TBD	2	TBD	1.62	TBD	N/A	3.24	TBD	TBD	TBD	TB	N/A	
Light-Absorbing Black-Out Paper, Plain Back, 27" x	TBD	1	TBD	33.90	TBD	N/A	33.90	TBD	TBD	TBD	TB	N/A	
Budget	$300.00												
Total Cost	$285.30												
Difference	$14.70												

End of Report