Periodontal Monitoring Device

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Advisor: Dr. Hall

Team Members

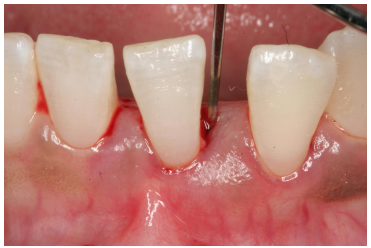
- Lindsey Cabanas
  - Mechanical Component
  - Record Keeper
- Naina Iyengar
  - Chemical Component
  - Budget & Timeline Manager
- Sindhuja Kuchibhatla
  - Electrical component
  - Project Leader & Webmaster

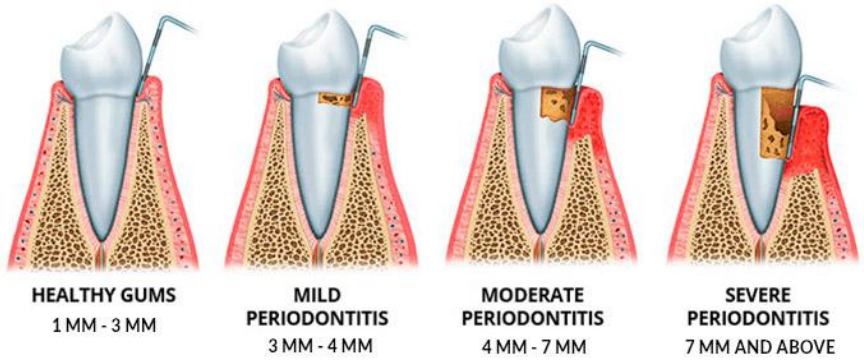
Periodontitis

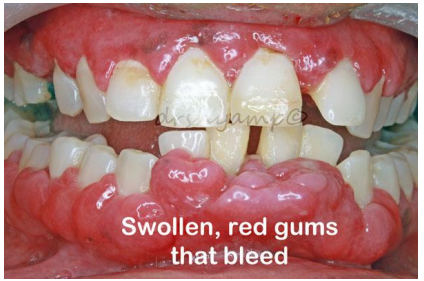
- Inflammatory condition that affects 80% of the adult population[1]
- Accumulation of bacteria between gingival tissues and teeth
- Results in attachment and bone loss[1]
- Increased pocket depth leads to tissue destruction

Diagnosis:

- Visual Inspection
- Measurement of pocket depth and attachment loss
- Observation of bleeding upon probing







Existing Devices

- Microfluidic chip-based immunoassay for TNF-α, IL-6, and CRP[2]
  - Binary; does not provide information about severity
- MyPerioPath[3]
  - Gives concentration of specific perio-pathogenic bacteria
  - Salivary diagnostic tool
  - Does not report severity of the disease
- Salivary Test from Columbia University[4]
  - For β-glucuronidase
  - Under development

Problem Statement

Since severe periodontitis occurs globally in 15-20% of adults aged 35 to 44[5], the conventional probing procedure is time-consuming and painful for the patient, and comparable devices diagnose rather than monitor disease progression, we are proposing a Periodontitis Monitoring Device for dental professionals, which will measure a clinically-relevant salivary biomarker and correlate it to periodontal severity (healthy, mild, moderate, or severe periodontitis).

Target Market

Direct Users: Dentists

Indirect Users: Patients

Problems

- Once periodontitis process is initiated, destruction is not predictable
  - Occurs in random bursts of activity during relatively short periods of time[6]
  - Necessary to provide treatment quickly
- Current probing procedures are painful
- Other procedures require samples sent to a lab

Device Solutions

- Allow monitoring of periodontitis at multiple stages
  - Display severity level of disease
  - Monitor response of disease to treatment
  - Predict prognosis of disease
- Non-invasive technique
  - Salivary test
  - Collection can be repeated with minimal discomfort
- Point-of-care device

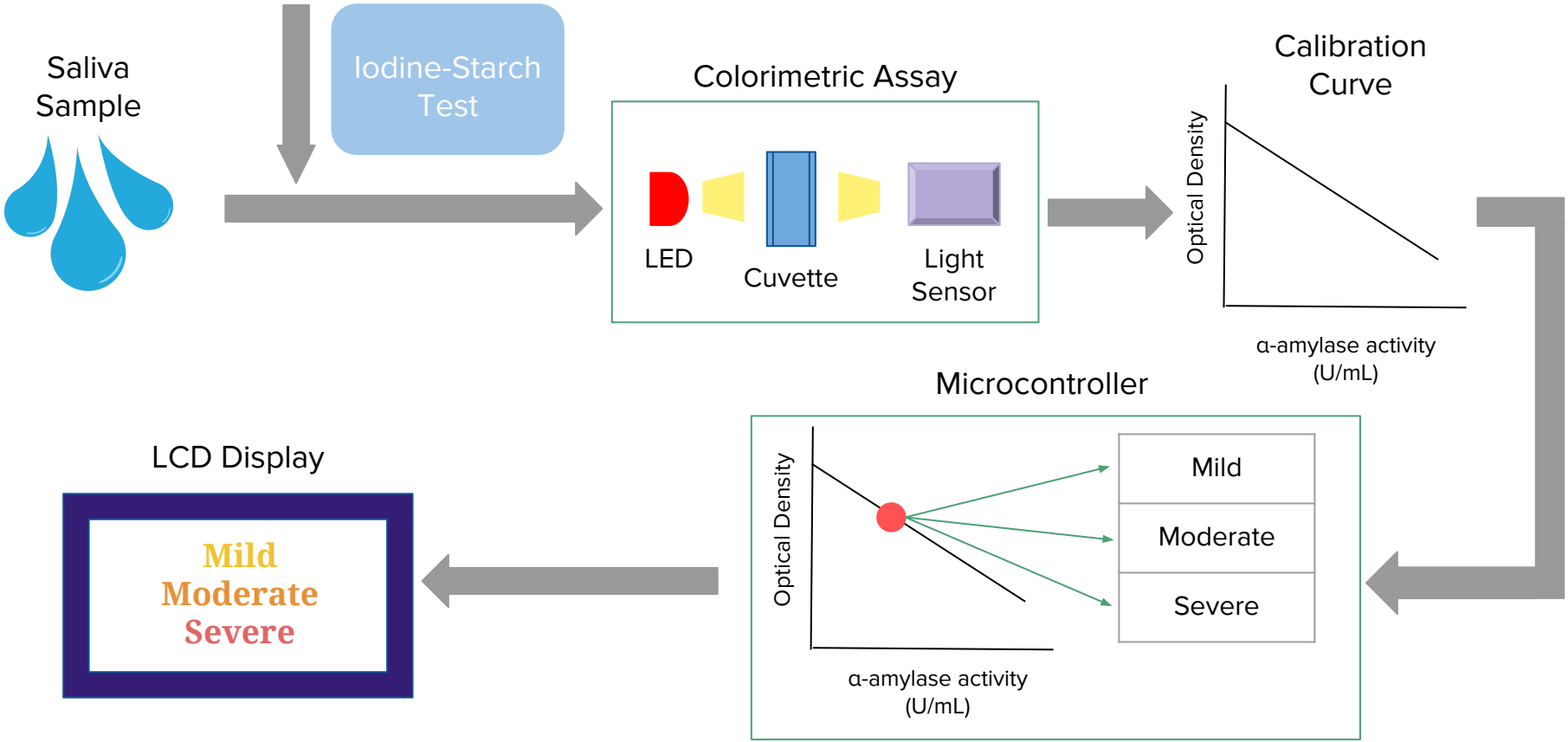
Main objective: Classify concentration of salivary biomarker to reflect severity level of disease

Biomarker: α-amylase

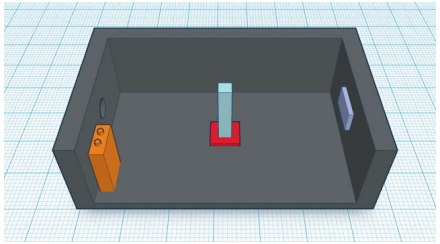
- Naturally found in saliva to break down large carbohydrates into smaller sugars
- Increased concentration with progression of periodontal disease
- Maintains mucosal immunity
    - Anti-microbial properties
- Increased response of salivary glands = increased synthesis and secretion of α-amylase[7]
    - Enhances oral defense mechanism
- Growth inhibitory activity against *Porphyromonas gingivalis* species
- Interferes with the aggregation, adherence, and biofilm formation of *Aggregatibacter actinomycemcomitans*[8]
- Cost-effective

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

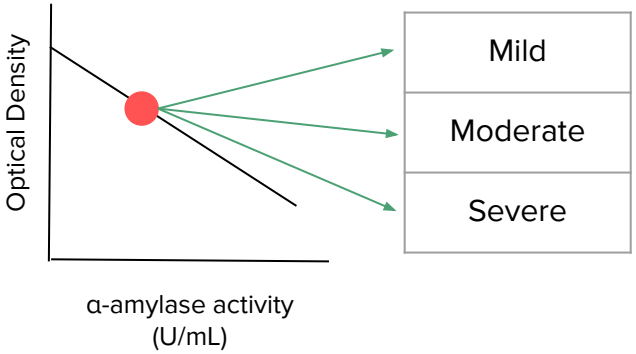
Overview of Design Solution



Design Input

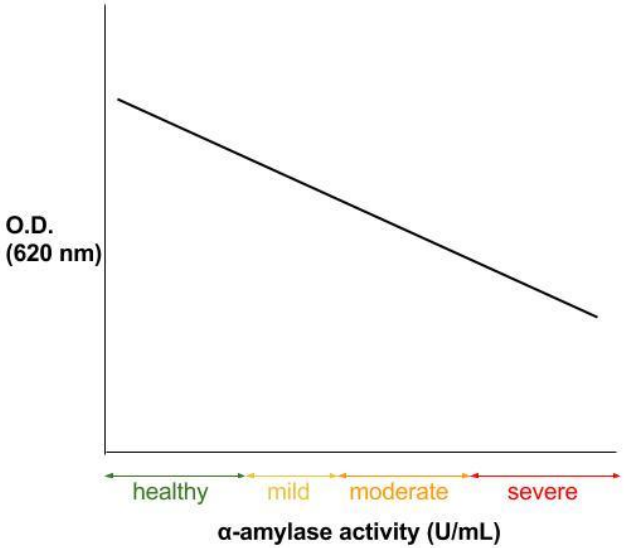
Component	Requirement	Specification
Overall Device 	Device must measure a salivary biomarker that relates to periodontal disease progression	Device must measure α-amylase activity in the saliva in a range from 86.31 U/L to 139.5 U/L[9]
Saliva Sample 	Device must require a small sample volume of saliva	Device must be able to measure the selected biomarker with a minimum 0.82 mL of saliva

Design Input

Component	Requirement	Specification
Microcontroller 	Device must classify concentration of biomarker as severity level	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)[9]
LCD Display 	Device must present the severity level of the disease to the user	Device must use an LCD display to relay the severity level (mild, moderate, or severe)

Chemical Component

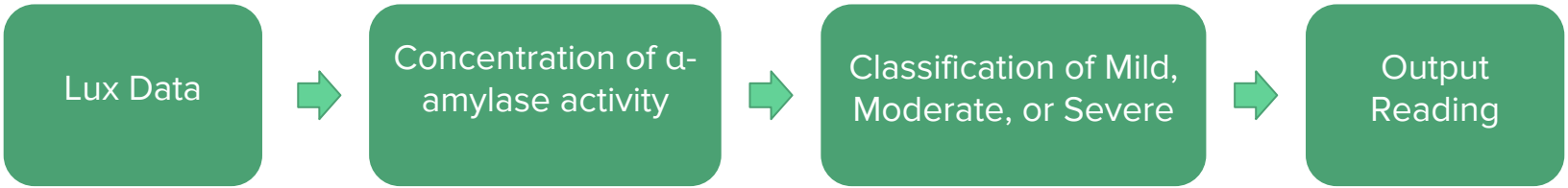
- starch: amylose and amylopectin
  - β-coils of amylose “trap” I_5^- molecules[16]
    - decrease in O.D.: blue-black light ➔ yellow light
      - Beer’s Law: A = e x b x C
- α-amylase from *Aspergillus oryzae*[17] is structurally similar[18] to human salivary α-amylase
- protocol: fungal α-amylase in PBS[19] ➔ potato starch for 1 min. ➔ HCl stop solution[20] ➔ I_5^- solution ➔ 620 nm
  - find conversion factor from mg to U
  - final standard curve: O.D. ➔ severity[10]



Reaction ➔ Measure Light Intensity ➔ Calculate Concentration of α-amylase ➔ Determine Severity Level

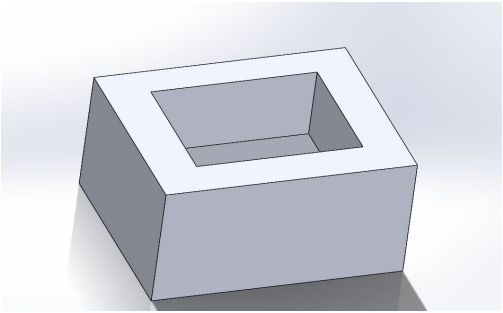
Electrical Component

- **LED Light & 9V Battery:** Red LED with 640 nm wavelength
- **TSL2561 Luminosity Sensor:** 0.1-40,000 Lux, configurable integration times and gain
- **Arduino Uno Microcontroller:** Relating the Lux data to concentration and categorizing the concentration into mild, moderate, or severe
- **LCD Display:** Output the level of severity to user

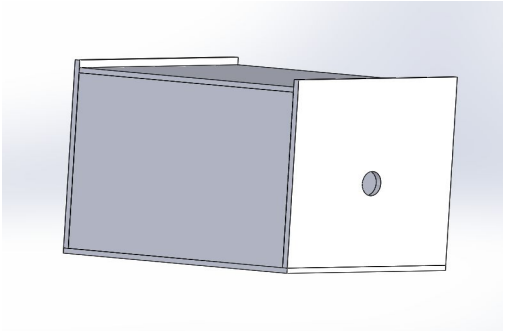


Mechanical Component

- Cuvette Holder
  - Raise the cuvette to the proper line of light
- Black Box
  - Made of black “chemical-and wear-resistance acetal”
    - a resistance to chemicals such as solvents and alcohols
  - Cleaned with a primer, and then held together by glue
  - Encasing the cuvette, cuvette holder, LED light, and the light sensor
- Platform
  - Will keep all of the components of the black box, the LCD, Arduino microcontroller off of the table top

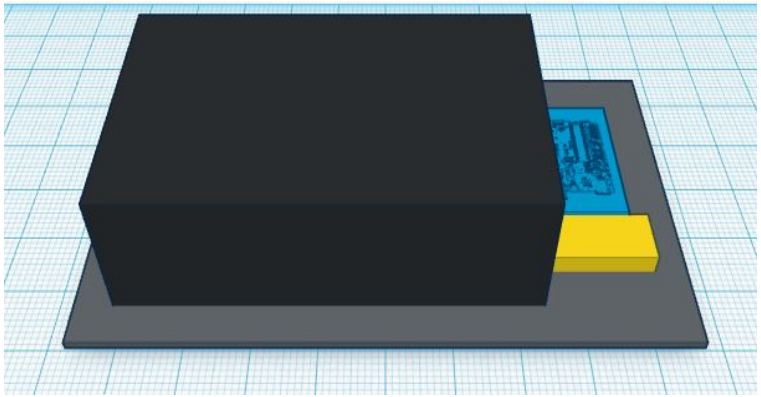


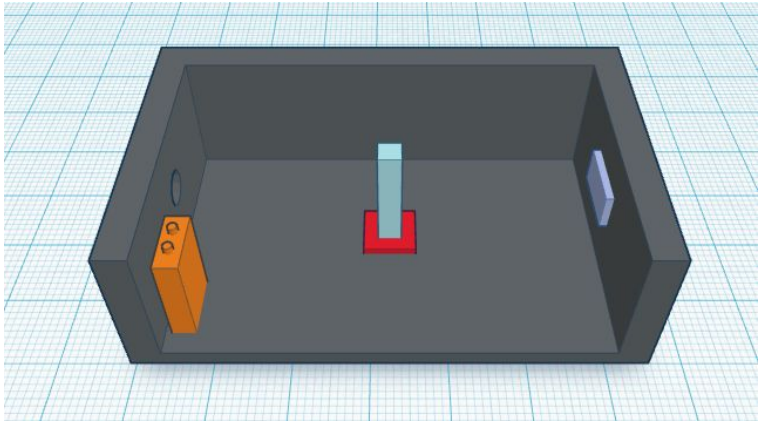
Dimensions:
Indentation: ⅛”
thickness with ½ “
depth
square ½ “ x ⅖ “



Dimensions:
5”x 5”x 6”

Prototype Design





Dimensions:

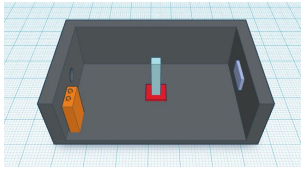
- **Black Box**: 5”x 5”x 6”
- **Cuvette Holder** : Indentation: 0.125” thickness with 0.5 “ depth square 0.5“ x ⅖ “
- **Cuvette**: 0.5” x 0.4“ x 1.75“
- **Arduino Board**: 2”x2.1”x 0.2”
- **Light Sensor**: 0.39” 0.29”x 0.49”
- **Battery**: 1.9"x1.0"x0.68"
- **LCD**: 0.9" x 2.7"x0.33”

Verification Activity

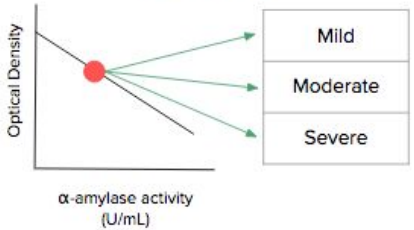
Ten solutions of varying known concentrations of biomarker will be made and tested within the device to confirm that the device accurately correlates the concentration to the severity level.

Component

Overall Device



Microcontroller



Requirement	Specification
Device must measure a salivary biomarker that relates to periodontal disease progression	Device must measure α-amylase activity in the saliva in a range from 86.31 U/L to 139.5 U/L[9]
Device must classify concentration of biomarker as severity level	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)[9]

Verification Activity

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.

Component

Saliva Sample

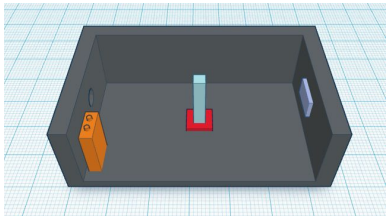
Requirement	Specification
Device must require a small sample volume of saliva	Device must be able to measure the selected biomarker with a minimum 0.82 mL of saliva

Verification Activity

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.

Component

Overall Device



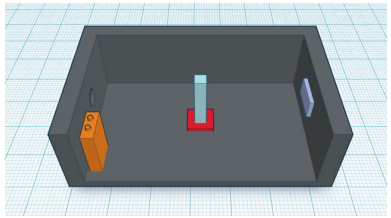
Requirement	Specification
Device must not be susceptible to corrosion or degradation caused by the reagents used	1. Device walls must be chemically inert materials 2. Reagents housed inside the device must not cause any degradation or corrosion for one week

Verification Activity

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.

Component

Overall Device



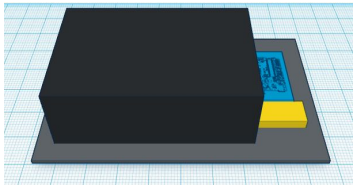
Requirement	Specification
Device must allow samples collected to be transferred with ease	1. There should be no loss of specimen volume while inputting the sample into the device 2. Inputting the sample into the machine should take no more than 2 minutes +/- 30 seconds

Verification Activity

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.

Component

Overall Device



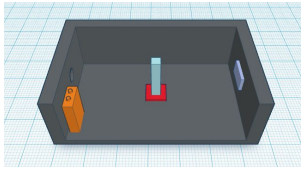
Requirement	Specification
Device parts/containers that come into contact with saliva must be resistant to moderate cleaning without impacting function of the device before use for each new patient	Device parts must be able to withstand cleaning from solutions with 5.25-6.15% sodium hypochlorite (corrosive cleaning agent in bleach) depending on the manufacturer

Validation Activity

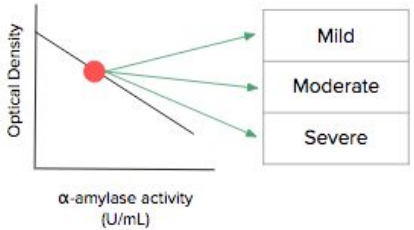
Simulated saliva samples (5 of each severity level) will be made. Samples will be entered into the device and run through the entire process. Final results from the LCD screen, the standard curve, and the known concentrations entered will be compared.

Component

Overall Device



Microcontroller



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Timeline

Fall Semester

- **Aug:** outlined functions of device
- **Sep:** brainstormed biomarkers, discussed assay types, first presentation
- **Oct:** selected biomarker and assay system, interim presentation
- **Nov:** drafted preliminary chemical protocol, codes, and dimensions
- **Dec:** final presentation SP I

Spring Semester

- **Jan:** optimize reagent volumes, write codes, finalize design plans
- **Feb:** create standard curves, make electrical schematics, begin machining, submit NEBEC abstract
- **Mar:** begin validation, verification of Requirements 1 & 5, begin affixing electrical parts, finish machining, interim presentation
- **Apr:** finish validation, verification of Requirements 2-4 & 6-9, NEBEC
- **May:** final presentation SP II

Citations

[1]Kim, Jeffrey J., Christine J. Kim, and Paulo M. Camargo. “SALIVARY BIOMARKERS IN THE DIAGNOSIS OF PERIODONTAL DISEASES.” *Journal of the California Dental Association* 41.2 (2013): 119–124.

[2]Herr, Amy E., Hatch, Anson V., Giannobile, William V., Throckmorton, Daniel J., Tran, Huu M., Brennan, James S., and Singh, Anup K. Integrated Microfluidic Platform for Oral Diagnostics. Annals of the New York Academy of Sciences. 1098. 362-374. 2007.

[3]OralDNA® Tests. Periodontal Testing. N.p., n.d. Web. 12 Oct. 2015. <http://www.oraldna.com/periodontal-testing.html>.

[4]"Technology Ventures." Saliva Test Detects Periodontal Disease. Columbia Technology Ventures, n.d. Web. 13 Oct. 2015. <http://innovation.columbia.edu/technologies/625_saliva-test-detects-periodontal-disease>.

[5]Oral Health. Fact sheet N°318. World Health Organization. April 2012.

[6]Goodson, J.M. Clinical measurements of periodontitis. *J Clin Periodontol. 1986 May; 13(5):446-60.*

[7]Baik JE, Hong SW, Choi S, Jeon JH, Park OJ, Cho K, Seo DG, Kum KY, Yun CH, and Han SH. Alpha-amylase is a human salivary protein with affinity to lipopolysaccharide of Aggregatibacter actinomycetemcomitans. *Mol Oral Microbiol.* 2013 Apr; 28(2):142-53.

[8]Acquier, Andrea Beatriz, Pita, Alejandra Karina De Couto, Busch, Lucilla and Sanchez, Gabriel Antonio.Comparison of salivary levels of mucin and amylase and their relation with clinical parameters obtained from patients with aggressive and chronic periodontal disease. *Journal of Applied Oral Science*, *23*(3), 288-294.2015.

[9]Cheesborough, Monica. Clinical Chemistry Tests. District Laboratory Practice in Tropical Countries, Part 1. Cambridge University Press. 362-364. 2005.

[10]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.

[11]Bhadane, Pravin K. Development of Microcontrolller Based Analyzer for the Detection of Chlorine in Water. International Journal of Research in Science, Engineering and Technology. 3(4). 10890-10896. 2014.

Citations (cont.)

[12]Crouch, D.J. (2005). Oral fluid collection: The neglected variable in oral fluid testing. Journal of Forensic Science International, 150(2-3): 165-173.
[13]Gram Iodine, Laboratory Grade, 100 mL Material Data Safety Sheet. Carolina Biological Supplier Company. http://www.carolina.com/teacher-resources/Document/msds-gram-iodine/tr-msds-gramiodineghs.tr
[14]Hazardous Waste Regulations. United States Environmental Protection Agency. http://www.epa.gov/wastes/laws-regs/regs-haz.htm
[15]Van Winkelhoff, A. J., and Boutaga, K. Transmission of periodontal bacteria and models of infection. Journal of Clinical Periodontology. 32(6). 16-27. 2005.
[16]Senese, F. How does starch indicate iodine? General Chemistry Online. (2015). <http://antoine.frostburg.edu/chem/senese/101/redox/faq/starch-as-redox-indicator.shtml>
[17]Machida, M., Yamada, O., and Gomi, K. Genomics of Aspergillus oryzae: learning from the history of Koji mold and exploration of its future. DNA Res. (2008) 15(4): 173-83. <http://www.ncbi.nlm.nih.gov/pubmed/18820080>
[18]Brayer, G.D., Luo Y., and Withers, S.G. The structure of human pancreatic α-amylase at 1.8 A resolution and comparisons with related enzymes. Protein Science. (1995) 4: 1730-1742. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2143216/pdf/8528071.pdf>
[19]Andrysewicz, E., Mystkowska, J., Kolmas, J., Jalbrzykowski, M., Olchowik, R., and Dabrowski, J.R. Influence of artificial saliva compositions on tribological characteristics of Ti-6Al-4V implant alloy. Acta of Bioengineering and Biomechanics. (2012) 14:4 <http://www.actabio.pwr.wroc.pl/Vol14No4/9.pdf>
[20]Rangunathan, R. and Swaminathan, K. Growth and amylase production by Aspergillus oryzae during solid state fermentation using banana waste as substrate. Journal of Environmental Biology. (2005) 26(4):653-6. <http://www.ncbi.nlm.nih.gov/pubmed/16459551>

Any Questions?

Design Input	
Device Requirements	**Device Specifications**
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[9]
2. Device must classify concentration of biomarker as severity level[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)[11]

Justifications:

[1]α-amylase increases in relation to the progression of periodontal disease[10]

[2]Ranges of salivary α-amylase concentrations for the different severity levels have been found by previous studies[10]

Device Requirements	Device Specifications
3. Device must present the severity level of the disease to the user	3.1 Device must use a microcontroller to relay the severity level (mild, moderate, or severe)
4. Device must require a small sample volume of saliva[4]	4.1 Device must be able to measure the selected biomarker with a minimum 0.82 mL of saliva
5. Device must not be susceptible to corrosion or degradation caused by the reagents used[5]	5.1 Device walls must be chemically inert materials 5.2 Reagents housed inside the device must not cause any degradation or corrosion for one week
6. Device sample chamber and samples used for testing must be able to be safely discarded[6]	6.1 No additional protective gear should be necessary to handle the chemicals apart from regular dentist gloves

Justifications:

[4]Current commercial oral fluid devices collect an average of 0.82-1.86 mL of saliva[12]

[5]Incompatible materials for iodine include water-reactive materials, metals (ferrous), rubber, and plastics[13]

[6]Guidelines are set by the EPA and FDA on how to properly dispose of chemicals that could be corrosive or considered biohazardous waste[14]

Device Requirements	Device Specifications
7. Device must allow samples collected to be transferred with ease[7]	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
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[8]	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
9. Materials for the device must cost less than the budget allotted by the engineering department[9]	9.1 All materials for the device must cost below $300 + possible additional funds
10. Device must be manufactured with existing equipment and facilities[10]	10.1 Existing equipment and facilities are those found in Armstrong Hall or TCNJ Science Complex

Justifications:

[7]Ease of use is dependent upon putting the sample into the device without it being a time-consuming activity

[8]Transmission of periodontal pathogens (Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis) can occur in adult individuals[15]

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

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