REDOX PROPERTIES, THIOLS AND PROTEIN CONTENT IN STIMULATED AND UNSTIMULATED SALIVA

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INTRODUCTION

- Saliva is predominantly water, making up around 99% of its composition.
- In the resting (unstimulated) state, approximately two-thirds of the total volume of the whole saliva is produced by submandibular glands. Upon stimulation, the parotid glands are responsible for at least 50% of the total volume of saliva from the mouth.

Iorgulescu, G. "Saliva between normal and pathological. Important factors in determining systemic and oral health." J Med Life 2(3): 303-307. (2009). pubmed.ncbi.nlm.nih.gov

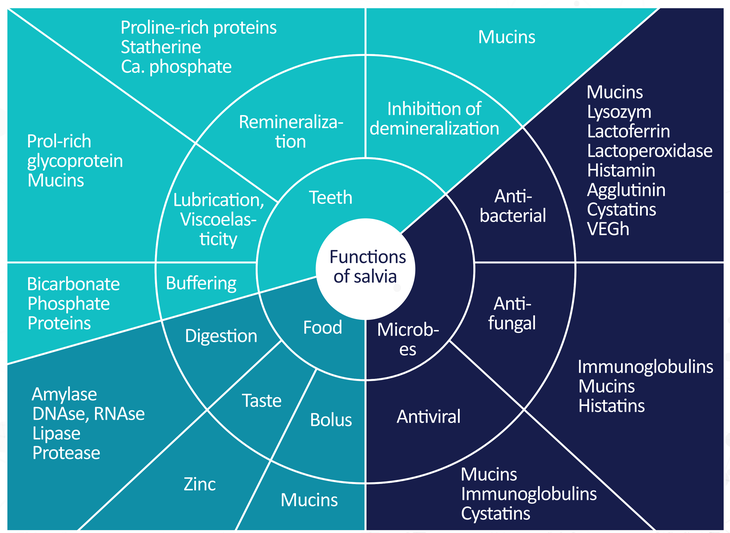

Figure 1

Scarano, E., et al.. "Proteomics of saliva: Personal experience." Acta otorhinolaryngologica Italica : organo ufficiale della Società italiana di otorinolaringologia e chirurgia cervico-facciale 30: 125-130. (2010) researchgate.net

GOALS AND AIMS

- The objectives of this research is to indicate how we can measure the total protein and thiol concentration within saliva using standard calibration curves achieved by spectrophotometry and spot if there is a difference in protein concentration between stimulated and unstimulated saliva.
- Bradford's assay is used to measure total protein concentration, while Ellman's assay is used to measure the concentration of thiols.

METHODOLOGY

WHAT OTHER STUDIES INDICATE

- Salivary proteins have been shown to be increased in medically compromised patients whose general conditions get worse.
- There can be many different reasons for decreased concentration of proteins in saliva.

Group	Total protein concentration
Adults	1.2 mg/ml ± 0.4 mg/ml
Adults fasting for 12 hours	0.8 mg/ml ± 0.8 mg/ml
Hospitalized elderly	2.3 mg/ml ± 1.0 mg/ml

Tab. 1

Rantonen, P. "Salivary Flow and Composition in Healthy And Diseased Adults." 72-75 (2003) citeseerx.ist.psu.edu

THIOLS

- In a study, 2 groups of people were chosen for measurement of salivary thiols. Group A are patients with periodontal pocket less than 5mm depth, whereas group B are patients with a pocket deeper than 5mm. Controls are healthy with no underlying conditions.

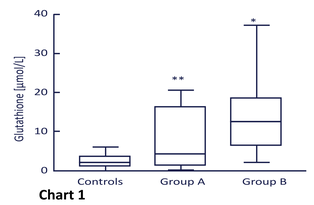

Chart 1

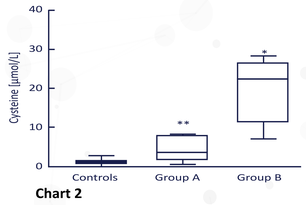

Chart 2

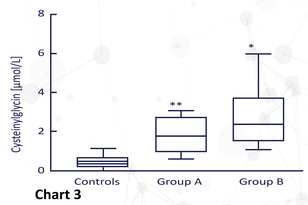

Chart 3

Zappacosta, B., et al. "Salivary thiols and enzyme markers of cell damage in periodontal disease." Clinical Biochemistry 40(9): 661-665. (2007). sciencedirect.com

EXPERIMENT I – BRADFORD'S ASSAY

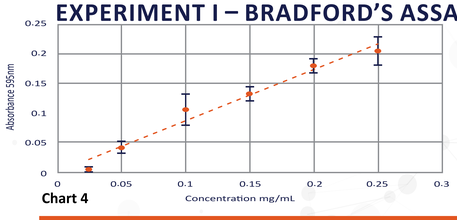

Chart 4

EXPERIMENT II – ELLMAN'S ASSAY

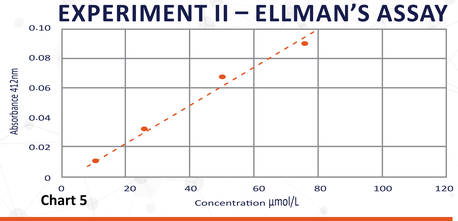

Chart 5

RESULTS

Experiment I	Absorbance minus blank
Sample 1	0.168367
Sample 2	0.0978

Tab.2

The samples of saliva were collected from the same person in fasting condition with first sample being unstimulated and second one stimulated and both were diluted 1:1 using physiological solution.Samples 3 and 4 are non diluted saliva from experiment I.
Y=(0.8675)X - 0.0002
Concentration of Saliva1: 0.1942 x 2 = 0.3884 mg/ml
Concentration of Saliva2: 0.1129 x 2 = 0.2258 mg/ml

Experiment II	Absorbance minus blank
Sample 1	-0.006034
Sample 2	-0.0015
Sample 3	-0.0027
Sample 4	-0.004467

Tab. 3

To minimize interference from mucins in saliva we can do: 1. Pretreatment of the saliva through selective precipitation, etc. 2. Selective blocking of mucins using reagents. 3. Control experiments with known concentrations of mucin.

CONCLUSION

Saliva is a solution that contains markers that can indicate pathological states in humans. Dramatic increase in concentration of thiols in saliva can be a sign of periodontal disease and may represent an adaptive response aimed at mitigating the detrimental effects of oxidative stress on the periodontal tissues. Concentration of salivary proteins may vary in stimulated, unstimulated and fasting conditions. It also increases in patients with underlying medical conditions due to inflammatory processes in the oral cavity that leads to leakage of plasma proteins into the oral cavity.