The effect of propolis mouthwash on the microcirculatory response to a reactive hyperaemia test

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Background: A rapidly expanding area or research is the link between oral health and cardiovascular health. For example, nitrite produced by oral bacteria can increase nitric oxide availability, a potent signalling molecule that regulates the vascular tone[1]. Chlorhexidine (CHX, Corsodyl™) has been used for several decades in dentistry to manage gum infections due to its powerful antibacterial properties. However, new evidence has shown that, besides pathogenic bacteria, chlorhexidine can also harm 'good' bacteria such as nitrate-reducing species, which are essential to sustain nitric oxide (NO) availability, and can challenge blood pressure management [2,3]. Hence we are seeking novel antibacterial products that are effective against pathogenic bacteria, whilst preserving 'good' bacteria (nitrate reducing species).

Propolis is wax-cum resin substance produced by honeybees and a natural source of phenolic compounds with potential prebiotic effects that can promote a positive shift in the composition and activity of oral bacteria. Recent studies have shown that propolis has a powerful antimicrobial effects against pathogenic bacteria, but its effect on nitrate-reducing species have not been investigated[4,5]. To the best of our knowledge this is the first study investigating and comparing the effect of propolis and chlorhexidine mouthwash on the abundance and activity of nitrate-reducing bacteria in regards to nitric oxide availability and cardiovascular health in healthy individuals.

Aims: This summer studentship aimed to compare the effects of a 7 day twice daily treatment with propolis (n-=23) and chlorhexidine (n=20) mouthwash on blood pressure and the microcirculation, in healthy individuals as part of an ongoing human clinical trial. I completed the final 10 patients in each group. The hypothesis was that phenolic compounds of propolis mouthwash could enhance activity of nitrate-reducing bacteria and nitrite availability, leading to improved microvascular function. On the other hand, chlorhexidine mouthwash could impair microvascular function due to its detrimental effect on nitrate-reducing activity and nitrite availability.

Methods: My role was to utilise my clinical skills gained in dental school thus far, by transferring my oral examination methods (soft and hard tissue examinations, basic periodontal examinations (BPE) and then a plaque and bleeding index) to a clinical trial environment. I gained new skills collecting and processing gingival crevicular fluid (GCF), saliva and nitrate mouth rinse samples for later oral microbiome analysis. I also centrifuged the samples and ensured that they were labelled and stored appropriately.

Important to this project I also undertook a reactive hyperaemia test in the human participants, before and after the mouthwash intervention as indicated previously[2]. Briefly, levels of oxygenated haemoglobin (HbO2) and deoxyhaemoglobin (HHb) on the left forearm (extensor digitorum) were continuously recorded using a near-infrared spectroscopy (NIRS) device (NIRO-200NX, Hamamatsu, Japan) at an output frequency of 1 Hz. The NIRS probe was secured with an elastic tensor bandage wrapped around the forearm to minimize movement and light intrusion. After baseline measurements (2 min) an automatic pneumatic cuff (Hokanson E-20 AG101, USA) was inflated ~5cm above the elbow for 5 min to an occlusion pressure of 200 mmHg. Then, inflation of the cuff was rapidly released and the NIRS measurements were continuously monitored for 5 more minutes. The average values during the 2 min baseline, 5 min occlusion, and 5 min recovery period were analysed. For the latter, I inputted and analysed and the data from the near-infrared spectroscopy device (NIRS), to determine whether our intervention affected microvascular function.

Results: We are yet to analyse and process all of saliva samples, but we do have preliminary results regarding the microvascular response. The microvascular function did not change between the pre and post-treatment test in the chlorhexidine group (Figure 1). In the propolis group, we found a trend for a decrease in the tissue oxygenation index (TOI) at the end of the occlusion post-treatment (from 48.7% to 46.4% +/- 16.2%), compared to pre-treatment (P=0.054). This could be related to oxygen diffusion from the blood vessel to the cell and mitochondrial function. There was also a trend to an increased ramp (difference between the lowest TOI value at end of the occlusion and peak value during reperfusion) between pre and post-treatment in propolis group, suggestive of an improved microvascular response from enhancement of nitrate reduction bacteria and more NO which modulates vasodilation, but this was not statistically different. Alongside these data we also detected that O'Leary plaque scores (%PI) reduced with use of both 7 days of chlorhexidine and propolis mouthwash ($p<0.05$), but to a greater extent with chlorhexidine. Bleeding on probing (%BOP) however, reduced with propolis mouthwash only ($p<0.05$), suggesting a reduction in gingival inflammation alongside the systemic microvascular responses.

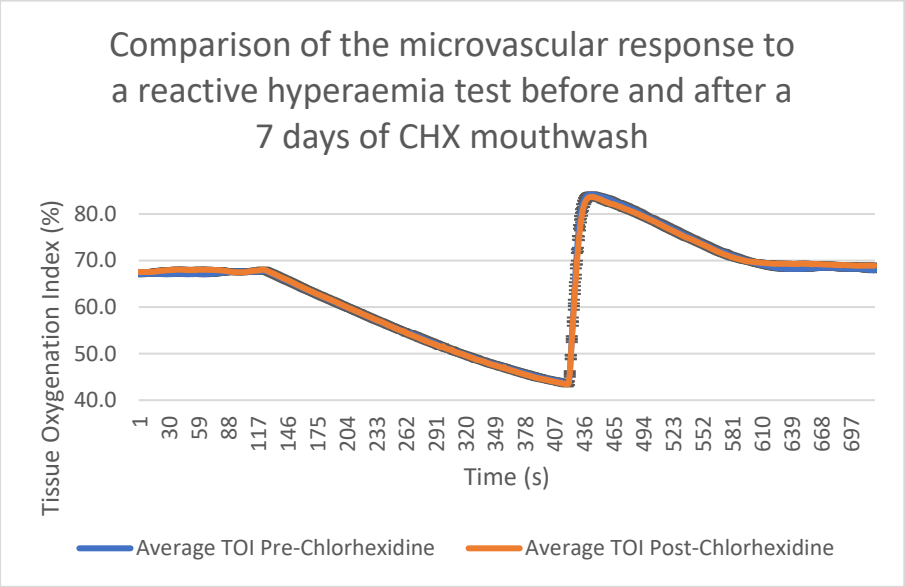

Figure 1

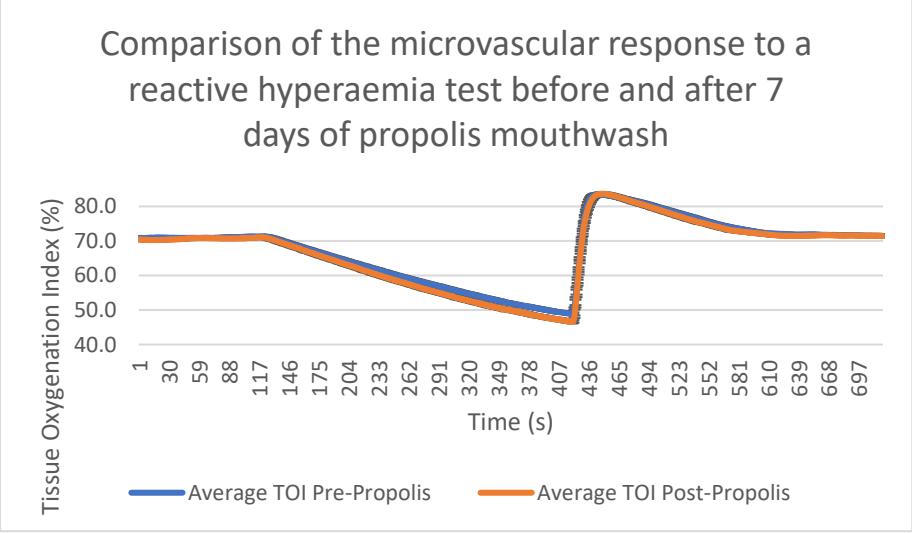

Figure 2

Conclusion and relevance to career: The preliminary data suggests that propolis mouthwash has the ability to decrease the PI% (albeit not to the extent of chlorhexidine) and BOP% in patients, without compromising the microvascular function, likely through not eradicating oral nitrate-reducing bacteria. On the other hand, chlorhexidine mouthwash did decrease the PI%, but not bleeding on probing, while having a neutral effect on the microvascular function.

There is still some analysis to be completed before final presentation, including analysis of the oral microbiome, but data collected from this study will hopefully contribute to the development of dental therapeutics that can target pathogenic bacteria, without eradicating bacteria essential for the host such as the nitrate-reducing species. The next step within this field would be to apply these findings to a population suffering from active periodontal disease and under high cardiovascular risk. We also plan to recruit a few more participants to gain more data to ensure our findings are statistically significant and align with the initial power calculation of n=25 in each group.

This opportunity has benefited me greatly and I have developed a better understanding of what is involved in research, with a plethora of skills that I will be able to utilise in the future. I am now considering an intercalated research MSc next year. I am very grateful for the opportunity to allow me to develop these skills and gain more experience in an area which really interests me.

Acknowledgements: I am very thankful to have been awarded the BMVBS summer studentship which I completed at the University of Plymouth within the Oral Microbiome Research Group. I have been interested by the oral microbiome and its impacts on systemic health since the start of my course in 2019, so being given the BMVBS Summer Studentship award, to achieve a greater understanding and investigate these links myself is an opportunity I will always be grateful for.

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