Study Design

The plaque and gingivitis inhibiting capacity of commercially available Essential oil - Listerine (alcohol containing and alcohol free) mouthwash, Dr Organic - Organic Aloe Vera mouthwash and non alcoholic 0.2% chlorhexidine mouthwash (Savacol) and 0.2% chlorhexidine (Savacol – alcohol containing) will be assessed by using a parallel randomized controlled single blind clinical study by utilizing the partial mouth gingivitis design. (Loe at al 1965).

Material and Methods

Patient selection

Participants will be males and females between 18-35 years old who will be thoroughly informed about the purpose and risks associated with the study.

Definition of Test and Control Groups

Test Groups

- Group A – Alcohol containing essential oil mouthwash (Listerine®)
- Group B – Non- alcohol containing Listerine
- Group C – Aloe Vera containing tea tree oil
- Group D – Non- alcohol containing Chlorhexidine (Colgate Savacol®)

Positive Control Group (Placebo group/comparator)

- Group E - Alcohol containing Chlorhexidine

Negative Control Group (Placebo group/comparator)

- Group F – Water

Pre-experimental period

- Visit 1 (14 days prior to commencement of the study)

Prior to commencement of the study all the participants will receive a professional dental clean. The purpose of this pre-experimental period is to achieve healthy gums.

Participants will also be advised to stop the use of their regular mouthwash (if any) for the entire pre-experimental and experimental period of overall 35 days.

Experimental period begins

Experimental oral hygiene protocol

- Participants will be required to clean all but the experimental teeth (Q1 or Q2).
- Participants will be required not to brush and floss their teeth in one of the upper quadrants (Q1 or Q2) depending on their handedness. Q1 – teeth on the upper right side and Q2- teeth on the upper left side. (Right handed dominant persons brush better in the upper left side (Q2) while left handed dominant persons are better at brushing the upper right side (Q1) (Park et al. 2006)).
- Right handed participants will be asked not to brush Q2 while left handed participants will be asked not to brush Q1.
- Participants will be asked to rinse their mouth twice daily with the allocated mouthwash.
- Participants will be advised not to use a toothpaste during the experimental period.

- **Visit 2 (Day 0)**

1. Time frame for each appointment 20mins
2. Allocation of participants into groups and issue of a mouthwash
3. Toothbrush, interdental brush and floss will be given to each participant
4. Participants will be advised to follow the experimental oral hygiene protocol
5. 5 different gum measurements (main variables) will be measured
6. Oral soft tissue health will be noted

- **Visit 3 (7days), Visit 4 (14 days)**

1. Time frame for each appointment 20mins
2. 5 different gum measurements will be measured
3. Oral soft tissue health will be noted

- **Visit 5 (21 days - end of experimental period)**

1. Time frame for each appointment 20mins
2. 5 different gum measurements will be measured
3. Oral soft tissue health will be noted
4. Participants will be requested to fill a questionnaire.
5. Participants will receive a professional dental clean to remove bacterial deposits and tooth staining
6. Participants will resume their routine oral hygiene practice (using their toothpaste) and stop the use of the allocated mouthwash.

Risks of the study

The general risks of participating in this study are:

- Tooth sensitivity for a few weeks
- Bleeding of the gums

The risks of using the mouthwashes are minimal

- Staining of teeth and the tongue
- Calculus (tartar) accumulation on the teeth
- Altered taste sensation
- Burning sensation of the oral mucosa while rinsing

Randomization

This study is a randomized controlled single blind clinical study. The associate investigator Dr Olivia Nova will be blinded to the treatment allocation as randomization into the six groups will be computer based and participants will be assigned a number.

The statistical calculation

Total Number of participants

- 15-20 participants in each of the Groups A, B,C,D,E,F
- 90-120 participants overall

Sample size was calculated from Marchetti et al (2011) who investigated the difference in plaque scores between an alcohol and non-alcohol based mouthwash between two groups. Using the means of 71.6 (±13.4) and 58.6 (±12.5) for the non-alcohol and alcohol based mouthwashes, respectively, a two-sided test, an alpha of 0.05, and power of 0.80, the sample size was determined as 16 participants per group.

The analysis plan

All data will be analysed using IBM Statistical Package for Social Sciences (SPSS) V21. Data will initially be analysed descriptively using graphs and tables. Crosstabs will be used to determine group percentages across the ordinal and nominal data measuring the levels of gingival inflammation (for example, periodontal and gingival indexes). Means and standard errors will be used for the parametric data obtained from the VAS measures in the self-report questionnaire.

Statistical significance will be calculated using $p < 0.05$. Differences between the groups will be determined using Kruskal Wallis test on the ordinal and nominal data of the gingival inflammation indices and ANOVA with multiple comparison (Bonferroni corrected) for the VAS measures.