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The repair of early tooth decay using a combination of stannous fluoride and a calcium milk peptide complex

Submission date	Recruitment status	[ ] Prospectively registered
20/11/2018	No longer recruiting	[ ] Protocol
Registration date	**Overall study status**	[ ] Statistical analysis plan
23/11/2018	Completed	[ ] Results
Last Edited	**Condition category**	[ ] Individual participant data
23/11/2018	Oral Health	[ ] Record updated in last year

Plain English summary of protocol

Background and study aims
Stannous fluoride and casein phosphopeptide amorphous calcium phosphate (CPP-ACP) have been shown independently to remineralise (repair) tooth enamel subsurface lesions (early tooth decay), but no study has investigated the two in combination. The aim of this study is to compare remineralization (repair) of tooth enamel lesions in situ using a combination of CPP-ACP and SnF2.

Who can participate?
Healthy staff and students of the University of Melbourne, aged 18-60

What does the study involve?
Human enamel pieces cut from extracted third molar (wisdom) teeth with artificially-created subsurface lesions (early tooth decay lesions) are prepared, sterilized and inserted into intra-oral palatal appliances (similar to removable partial dentures) that are worn by the participants. Participants rinse their mouth with one of five rinses for 60 seconds, 4 times per day, for 14 days. The five solutions are tested in a random order with a one-week break between solutions. The mouth rinse actives (SnF2, NaF and CPP-ACP) are commercially available in oral care products in Australia. Enamel lesion mineral content of the enamel pieces in the worn appliances is measured after each 14-day treatment in the laboratory after the enamel pieces are removed.

What are the possible benefits and risks of participating?
The expected benefit of the study is that it will provide important information on the relative effectiveness of these oral care actives. It is expected that all CPP-ACP and fluoride containing mouth rinses will be superior to the fluoride alone solutions in the remineralization of enamel subsurface lesions. There is very little risk for the participants. The solutions are safe for human use.

Where is the study run from?
University of Melbourne (Australia)

When is the study starting and how long is it expected to run for?
May 2014 to October 2014

Who is funding the study?
Department of Industry, Innovation and Science, Australian Government

Who is the main contact?
Prof. Eric Reynolds

Contact information

Type(s)
Scientific

Contact name
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Additional identifiers

Protocol serial number
University of Melbourne ID: 1441572

Study information

Scientific Title
The effect of rinsing with solutions containing sodium fluoride or stannous fluoride with and without casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on remineralisation of enamel subsurface lesions in situ

Acronym
CPP-ACP/SnF2 in situ clinical trial

Study objectives
Addition of CPP-ACP to SnF2 enhances tooth enamel lesion remineralization in situ.

Ethics approval required
Old ethics approval format

Ethics approval(s)
University of Melbourne Human Research Ethics Committee, 26/08/2016, ref: 1441572

Primary study design
Interventional

Study design
Single-centre double-blind randomized controlled cross-over design

Study type(s)
Treatment

Health condition(s) or problem(s) studied
Dental caries (tooth decay)

Interventions
The in situ study will be a randomised, controlled, double-blind cross-over study design. Artificially demineralised carious lesions were created on enamel blocks sectioned from human third molars and the blocks were each cut into experimental and control half-blocks as in the in vitro remineralisation model. Experimental half-blocks were inserted into intra-oral appliances to create a plaque retention site over the enamel lesions as described previously (Cochrane et al. 2012; Shen et al. 2018). Eight healthy subjects with an average age of 43 ± 11 years old (four males and four females) participated. It was calculated that eight participants was required to provide the required statistical power (90%, $p < 0.05$) based on previous investigations using a similar cross-over in situ model (Cai et al. 2009; Shen et al. 2018; Walker et al. 2010). The required sample size was calculated using the G*Power Version 3.1 sample size package (Faul et al. 2007) and was based on a repeated measures analysis of variance with five levels, an effect size of 0.97, a correlation, ρ, between any pair of treatment means of 0.5 and a non-sphericity correction ε of 0.5. The effect size of 0.97 was based on detecting differences between ΔZd-ΔZr means of 70 (CPP-ACP +NaF and CPP-ACP + SnF2) and a common standard deviation of 100 within groups. The non-sphericity correction adjusts for heterogeneity in the variances of the repeated measures. With a 5% significance level and a power of 90% at least six subjects were required. To allow for subject attrition eight subjects were recruited for the study.

Participants were randomly allocated to rinse with 5mL of one of the five remineralisation solutions for one minute, four times each day for 14 consecutive days (treatment period) as described below and previously (Shen et al. 2018):
1. 0.4% (w/v) CPP-ACP and 290 ppm F (220 ppm SnF2 and 70 ppm NaF)
2. 290 ppm F (220 ppm SnF2 and 70 ppm NaF)
3. 0.4% w/v CPP-ACP
4. 0.4% w/v CPP-ACP and 290 ppm F as NaF
5. 290 ppm F as NaF

Participants were instructed to continue their normal dietary regime during the study and were given a toothbrush and sodium fluoride toothpaste (1450 ppmF) to brush their teeth twice a day. The intra-oral appliances were removed during oral hygiene procedures and were kept in a sealed humidified container. Participants were also instructed to clean their intra-oral appliances with a toothbrush and a fluoride-free denture paste supplied to them, taking care to avoid the attached enamel half-blocks. At the conclusion of the 14 days, participants rested from the study for one week (washout period) then began another treatment with a randomly assigned treatment solution. This was repeated until participants had rinsed with all five solutions. During

the treatment periods subjects maintained a diary recording each rinse and duration of rinse. Following the treatment period, participants returned their appliance, empty rinse tubes and diary to the investigators and new enamel half-blocks were attached for the next treatment period. Researchers and participants were blind to the treatment code. An independent senior staff member held the treatment code securely which was only released after data collection and analyses. Each experimental half-block was paired with its control half-block following the remineralisation period for analysis of mineral change using transverse microradiography.

Intervention Type
Other

Primary outcome(s)
The effects of the rinses on enamel remineralization will be compared using integrated mineral gain/loss (ΔZd-ΔZr/%R) as the primary outcome measure. At the end of each treatment period each test half-slab will be paired with its control half-slab and embedded, sectioned and analysed by transverse microradiography to determine mineral content as described previously (Cochrane et al. 2012). Lesion parameters, lesion depth (LD) and integrated mineral loss (ΔZ) will be determined and the ΔZ value for the control demineralized lesion will be designated ΔZd and that for the treated lesion will be designated ΔZr. These values will then be used to calculate total mineral loss or gain ΔZd-ΔZr and percentage mineral change (%R) as (ΔZd-ΔZr/ΔZd) x 100. The change in lesion depth will be calculated as LDd-LDr.

Key secondary outcome(s)
The profile levels of fluoride and tin in enamel lesions remineralised measured using Electron Probe Microanalysis following exposure to the five solutions

Completion date
31/10/2014

Eligibility

Key inclusion criteria
1. Eight healthy adults living in Melbourne, Australia with a fluoridated (0.9 ppm F), reticulated water supply
2. Staff and students of the University of Melbourne
3. Age 18-60 years
4. At least 22 natural teeth
5. Unstimulated whole salivary flow rate of ≥ 0.2 ml/min
6. Gum-stimulated whole salivary flow rate ≥ 1.0 ml/min

Participant type(s)
Healthy volunteer

Healthy volunteers allowed
No

Age group
Adult

Lower age limit

18 Years

Upper age limit
60 Years

Sex
All

Key exclusion criteria
1. Currently using antibiotics or medications that may affect salivary flow rates
2. A history of severe oral disease

Date of first enrolment
20/05/2014

Date of final enrolment
06/06/2014

Locations

Countries of recruitment
Australia

Study participating centre
University of Melbourne
Level 6, 720 Swanston Street
Carlton
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Sponsor information

Organisation
University of Melbourne

ROR
https://ror.org/01ej9dk98

Funder(s)

Funder type
Government

Funder Name
Department of Industry, Innovation and Science, Australian Government

Alternative Name(s)
Department of Industry, Innovation and Science

Funding Body Type
Government organisation

Funding Body Subtype
National government

Location
Australia

Results and Publications

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study are/will be available upon request from Prof. Eric Reynolds. The data that will be provided are the enamel lesion parameter details. No identifying information will be provided on the volunteers who participated in the clinical trial, only general demographic details (e.g gender breakdown and average age of groups). The data are currently available. They will be published shortly and will be retained on record for at least 15 years. The raw data of the enamel parameters can be provided in an electronic format (spreadsheet) if requested. As no identifying or personal information on the participants will be disclosed there are no legal or ethical implications of disclosing the enamel parameter dataset.

IPD sharing plan summary
Available on request