Nobio Clinical Study - Demineralization Prevention With a New Antibacterial Restorative Composite

NCT04059250

September 10th, 2018

Protocol PROPOSAL

1. TITLE PAGE

Study Title:	Nobio In Situ Clinical Study – Pilot Study
Protocol Number:	Nobio-2018-08 – September 10th, 2018
Trial registration	ClinicalTrials.gov – registration is not necessary
Investigational Product:	Nobio quaternary ammonium silica dioxide antimicrobial nanoparticles in composite resins
Indication:	Antibacterial – antibiofilm effect on enamel adjacent to composite
Funding:	
Source of Funding:	
Secondary Sponsor:	
Development Phase:	Clinical in situ study, pilot
Sponsor's Responsible Officer:	
Sponsor Signatory:	
Effective Date:	

2. SYNOPSIS

Study Title: Nobio In Situ Clinical Study – Pilot Study	
Protocol number: Nobio-2018-08	
Principal Investigator: Peter Rechmann, PhD, DMD, Prof. Dr. med. dent. Department of Preventive and Restorative Dental Sciences, School of Dentistry, UCSF Sponsored Research, PI initiated	
Study center: University of California at San Francisco	
Study period: 10-2018 to 06-2019	**Phase of development:** Clinical In Situ Study, Pilot

Background

In research the use of experimental intra-oral caries models dealing with caries prevention has increased. The most realistic experimental model is the in vivo model that uses living teeth, followed by the in situ model using specimens with natural surfaces held in the mouth during the experimental period [1]. In situ models have the potential to study both fundamental aspects of the caries process as well as more applied research problems in caries prevention in human subjects without actually causing caries in the natural dentition [2]. In situ models are able to produce statistically significant differences to compare the use of different fluoride toothpastes and controls [3-9], fluoride mouth rinses [10], antimicrobial releasing agents [11], varnishes [12], and other fluoride releasing products [13-16]. In situ studies also may deal with the effect of biomaterials and food on demineralization and remineralization [17, 18]. A few in situ studies have been done related to root caries and its prevention [19-22].

Regarding the study design numerous in situ models have been described [2]. Orthodontic brackets have been used as a plaque trap on enamel [23, 24], as well as a carrier for slabs [25]. Interproximal models [26] with partial dentures for testing the effect of fluoride on enamel have also been used.

As a major issue in dentistry, it has been stated that two-thirds of all restorative dentistry involves the replacement of failed restorations [27]. Secondary caries indicates a lesion developing at the margins of an existing restoration and is the major reason for replacement of amalgam and composite resin restorations in operative dentistry [28, 29].

Two regions have been described when considering the process of secondary caries; the surface lesion, which develops perpendicular to the tooth surface and can be considered a primary lesion developing next to a restoration, and the wall lesion, which develops perpendicular to the tooth/restoration interface [30]. To simulate a failed interface between a tooth and a restoration, a gap can be formed between the two during restoration prior to a cariogenic challenge [31]. An in situ study with four different gap sizes (50 µm, 100 µm, 200 µm, and 400 µm and 1 non-bonded interface without a gap) showed no trend for a relationship between the lesion depth and the gap size [32].

A systematic review about simulation of secondary caries in in situ models lately reported that three main groups of in situ models could be identified by sample placement; 68% of the models placed samples palatally in the upper jaw, and the lower jaw model could be divided into the buccal (26%) and approximal areas (6%, only in edentulous patients). Average lesion progression in enamel per day next to composites was 4.3 +/- 2.8 µm (range1.1-8.8 µm/day). It was found that studies conducted with palatal models showed caries progression rates 2-5 times higher than the estimated clinical progression rates.[33] Up to eight dippings a day with concentration of 20% sucrose were used. Fluoride-containing toothpastes were used in 48% of the studies, and 42% had their volunteers use fluoride-free toothpaste. Lesion development was measured using microradiography techniques (TMR or transversal wavelength independent microradiography [TWIM]) in 36% of the studies, 48% used cross-sectional microhardness. Two main trends were observed, with 4 studies showing a rate of ≤2 µm/day and 7 studies showing a rate of >4 µm /day. These 7 studies with a high progression rate were all palatal models, used mesh for plaque promotion, and experiment durations of only 2 weeks. It was also observed that samples with a natural surface, a surface not polished flat, slowed down initial lesion formation [33].

The influence of dental materials to inhibit caries development has been tested evaluating for instance the caries inhibition effect of glass ionomer cement [34, 23], and the effect of glutaraldehyde on secondary caries [35]. It was shown that fluoridated composites might reduce or inhibit secondary caries. In an in situ model investigation the effect of F-releasing composites on enamel demineralization around an artificial gap of 200 microns width was quantified after 1 month [35]. In the gap, all fluoridated composites reduced the enamel demineralization significantly with respect to the non-fluoridated controls [35]. The reduction of the lesion depth for three different materials was 44, 46 and 27%, respectively, while the mineral loss reduction was 56, 54 and 25%, respectively [35].

There is an increasing clinical need to design novel dental materials that combine regenerative and antibacterial properties. A recently developed sol-gel-derived bioactive glass ceramic containing silver ions (Ag-BG) in the resin composite induced remineralizing and showed antibacterial properties [36]. The total bond strength of the new Ag-BGCOMP composites was not significantly affected [37].

As stated, secondary caries and in addition hybrid layer degradation are the two major challenges encountered in long-term resin-dentin bond stability. As a link between resin and dentin, adhesives that possess both antimicrobial and anti-proteolytic activities are in demand for eliminating bacteria-induced secondary caries and preventing hybrid layers from degradation. Lately a study reported that a new quaternary ammonium methacryloxy silane (QAMS) prepared from sol-gel chemistry was incorporated into experimental adhesives to examine their antimicrobial effect and anti-proteolytic potential [38]. Around the world several groups are researching the possibility of using composite restorative materials that incorporate antibacterial agents to overcome the described problems [37, 39-43].

Nobio has developed dental restorative materials with long term antibacterial properties in order to fight recurrent decay around restorations. These composites with incorporated non-leaching antibacterial agents might overcome the vicious circle of newly developed cavities around freshly placed fillings. The ongoing caries activity due to bacteria around the filling may be interrupted by quaternary ammonium silica dioxide (QASi) particles incorporated in the test composite at 1.2% (by weight). These particles are members of the Nobio

Antimicrobial Particle (NAP) family (Nobio, Israel). QASi are synthesized to form a high concentration of antimicrobial groups that are covalently-bound onto a carrier core, such as silica. The resulting micro or nano-sized QASi particles are mixed with other fillers of the dental composite material, during manufacturing. Following in situ placement and light-initiated polymerization, QASi particles are permanently retained in the final dental restoration. Laboratory studies have shown that the quaternary ammonium silica particles are potent antibacterial agents, do not leach out in contrast to other antimicrobials or caries preventive active substances, inhibit the breakdown of the composite, and maintain antibacterial activity over time [39-42].

Nevertheless, the key experimental parameters for a successful and valid in situ study are the panelists selection and appliance design, the type of hard tissue substrate and the method of assessing mineral status, and the overall study design and clinical protocol [2, 18, 44, 45].

Objective

The overall objective of this clinical pilot study is to prove in an in situ model how effective Nobio composites with antimicrobial activity reduce caries in enamel adjacent to the composite restorative material.

Hypothesis

The hypothesis to be tested is whether composites with Nobio's QASi antibacterial particles significantly reduce demineralization in adjacent enamel in comparison to a conventional composite in an in situ clinical study, with mineral loss evaluated by cross sectional microhardness testing in the laboratory.

Product under Investigation

The product under investigation is Nobio's light-cured composite restorative material, suitable for all applications from Class I through Class VI.

Study Design & Outcomes

The study is designed as an in situ study.

Primary outcome

1. Mineral loss ΔZ: Significant reduction in mineral loss ΔZ in the Nobio group compared to the control is expected.

Secondary outcome

2. Microbial plaque pH - level: A pH-indicator used on the composite slab immediately after the wearing period will check for the pH level of the microbial plaque on top of the composite slab after wearing; a neutral pH on the Nobio composite slabs in comparison to the control composite slabs is expected. Prior to pH-measurements the denture might be rinsed with 2% dextrose.

Methodology

The study is planned as an in situ study. Subjects, wearing partial dentures with acrylic flanges on both sides of the mouth will be recruited. On each side of the denture an enamel slab will be placed next to a composite, separated by a tiny gap. Placing a thin metallic matrix band (38 µm) between the enamel slab and the composite, before curing the composite, will create the gap. After curing, the matrix band will be removed. On one side of the denture the composite will be Nobio's antibacterial composite, on the other side a regular composite (a Nobio composite w/o particles or another commercial composite (3M / Dentsply) will serve as control material). Enamel slab and composite will be recessed into the flange, allowing microbial plaque to accumulate on top of it and especially in the gap. After a specific period of wearing the dentures with the slabs, decalcification - ΔZ mineral loss - in the enamel slabs adjacent to the gap will be determined by cross-section microhardness testing in the laboratory. Average ΔZ mineral loss will be calculated for the Nobio group and the control group, and difference will be tested for statistical significance. ΔZ mineral loss of 670 ±195 Vol%xµm for the active Nobio-composite and ΔZ of 1,200±450 Vol%xµm might be expected for the inactive control side. These predictions are based upon former studies related to fluoride use, but they are predictions based on a best guess as to what might happen.

A pre-study will be conducted, in which 3 to 5 subjects will be selected and will wear dentures with enamel slabs incorporated, as described above, to determine whether a wearing period of four weeks is enough to achieve sufficient demineralization of the enamel slab. Depending on the results, the data from these subjects might be included in the final data set. If the 4 week test period proves to be sufficient in this pre-study and the same protocol is used for the main study this will allow the pre-study subjects to be included in the main results. It is possible that a second pre-study will be needed, depending on the results of the first one. The second pre-study may potentially ask the same subjects to be involved, again.

Study Participants and Procedures

A total of about 20 subjects will be recruited into the study (20 subjects will allow for 2-3 drop-outs). Subjects will be recruited from the UCSF Dental Center and will be partial denture wearers with denture flanges on both sides of the mouth. Subjects will be selected according to specific inclusion and exclusion criteria (see below).

Subjects' age range will be from 18 to 80 years. We will select subjects, who are already wearing a lower removable partial denture with lateral teeth replacement. The natural dentition must have at least 8 remaining teeth to ensure typical caries bacteria colonization. The subjects must be healthy, and have healthy oral conditions. They are not allowed to show active caries lesions but should have a recent history of former caries. Subjects with acute periodontal diseases are excluded. The stimulated saliva flow rate will be measured. Subjects exhibiting saliva flow rates below 0.7 ml/min for stimulated saliva will be excluded (subjects with a saliva flow rate below 0.7ml/min are at extreme caries risk and are in need of special caries prevention efforts).

Subjects start their study involvement with a 2-week washout period using an Over-The-Counter (OTC) 1,100 ppm F-toothpaste to brush their teeth (there will be no professional tooth

cleaning at the study start in order to not interrupt biofilm activities). After the 2-week washout period, the slabs will be mounted into the denture flanges (test side: enamel slab adjacent to Nobio antimicrobial test composite; control side with enamel slab adjacent to inactive composite), and subjects will wear their denture for 4 weeks. After 4 weeks plaque pH-levels on top of the composite will be checked, enamel samples will be removed, and samples will be prepared for the laboratory cross sectional microhardness testing to determine the ΔZ mineral loss.

The participants will be instructed to brush their teeth twice daily for one minute with the provided OTC 1,100 ppm Fluoride toothpaste. The participants will be asked to remove the dentures from the mouth before brushing their teeth. They will be asked to brush the inside of the denture and the denture teeth but not the area with the slabs.

The subjects will be asked to fill in a log of their daily tooth-brushing schedule, and tubes of toothpaste for subject's use only will be distributed and weighed before and after each trial period to crosscheck compliance. The study coordinator will telephone the households approximately once a week to assist with compliance.

Recruitment and Consenting

Flyers posted in the UCSF Dental Center will be used to recruit patients and study investigators (and/or staff) will recruit their own patients directly in person (outside the direct treatment area). To achieve adequate participant enrolment to reach targeted sample size, advertisements will be placed in the Dental Clinic areas as well as in other patient areas of the UCSF School of Dentistry, Dental Center.

When patients are seen by their dentist and the treating dentist believes that the patient might fit into the study, he/she will ask the potential participant whether she/he might be interested in being in a dental study. If the subject is interested, the treating dentist will ask the potential participant whether she/he gives permission to disclose her/his name to the researchers. If the patient agrees, the Principal Investigator (PI) (Peter Rechmann) and/or the Clinical Study Coordinator (CSC) (Beate Rechmann) will ask the potential subject whether she/he is interested in participating in this study. If so, the Investigator/Clinical Study Coordinator will explain the whole study and will further check for eligibility.

Consenting all study subjects will take place prior to entering the study (written informed consent). Potential participants will be consented and enrolled by the PI and the CSC, respectively.

Blinding

The doctor performing the treatment and the study coordinator will not be blinded to which side of the denture will carry the test and which is the control slab. The participant will be blind to the treatment/control allocation side. For blinding of the laboratory person doing the microhardness testing – please, see the below section "*Blinding in the laboratory*".

Source of teeth: We will use teeth that have been previously extracted for clinical reasons (not research purposes) in the Dental Clinics of the School of Dentistry. Correct collecting, storing,

handling etc. of human teeth under biosafety aspects is covered under our BUA approval (BUA 2308-BU-01-INC) and a UCSF IRB exempt approval for collecting extracted teeth.

Test samples

Extracted teeth (molars) will be stored in 0.1% thymol solution in deionized water and sterilized with gamma irradiation (Cs 137) at a dose above 173 krad overnight. Following sterilization, the collection media will be replaced with fresh deionized water and thymol. The tooth roots will be removed below the cemento-enamel junction. The dental crowns will be cut in halves.

At least 60 enamel samples from 20 extracted molars will be prepared. The test surfaces will be cleaned, and the surface will be flattened by serial polishing (600 grit silicon carbide polishing paper followed by 6 µm diamond polishing suspension/or 1,200 grit polishing paper, sterilized). The smear layer will be removed by sonication. Three standard slabs approximately 3 x 2 x 2 mm will be cut from each tooth crown. One will be retained in the laboratory in a moist atmosphere in a sealed vial as an untreated control, and the adjacent two will be used in the subjects' mouths. All teeth will be sterilized with gamma irradiation before use in the mouth.

Testing and Analysis methods

The analysis method used will be cross sectional microhardness by detailed “scatter pattern” of indentation. At the end of the 4-week in situ wearing period treatment slabs and the control slabs will be removed from the denture flanges. Both slabs will be embedded and used for microhardness testing as described below.

Microhardness measurements
After the 4-week wear period the slabs will be embedded in epoxy resin (Ladd Research Industries), leaving the flat section surface uncovered. After serial polishing, the exposed flat surface will be indented (25 g weight) to test for microhardness cross-sectionally using a Buehler Microhardness tester (Buehler, Germany) and microscopic examination (Featherstone et al., 1983)[46]. The first indent will be placed 15 µm from the resin interface and 100 µm from the edge of the lesion. Subsequent indents are placed in 5 µm increments to a final depth of 50 µm in the underlying enamel; implementing a V-shaped pattern prevents interaction and interference between the indents.

Additional indents will be placed at 25µm intervals into underlying sound enamel following a straight line perpendicular to the outer surface to a depth of 300 µm. The volume percent mineral for each indent is normalized based on sound underlying enamel (100-300 µm) set at 85%. This is an internal calibration of the measurements that exists within the ΔZ formula, which allows for normalization of the microhardness data on a per-tooth basis, such that tooth-to-tooth variability is eliminated. All data will be verified for reproducibility of the measurement method and quality assurance by repeating any outlier measurements. Measurement of indentation lengths / demineralization will be calculated with the aid of Image pro plus 4.0 software which is used for capturing and measuring the image through a microscope (Olympus BX50, Melville, NY) at 500X magnification.

The overall relative mineral loss, ΔZ, for each sample is calculated by creating a hardness profile curve by plotting normalized volume percent mineral against distance from the outer

enamel surface. The area under the curve that represents ΔZ (μm x vol % mineral) is calculated using Simpson's integration rule. The individual ΔZ values for each lesion in each group will be combined to give a mean ΔZ and standard deviation for each of the groups (test, control).

Blinding in the laboratory

The technician measuring the indentation lengths will be blinded to the group assignment - test and control group, respectively. Slabs will be delivered to the laboratory technician in number coded vials. The CSC will have an appropriate list for coding and encoding.

Statistical methods – sample size calculation

The Nobio composite with antibacterial nanoparticles has not been tested so far in a clinical or in an in situ study. Consequently, there is no way to calculate what the effective sample size would be and how much demineralization/remineralization will occur.

We assume that based upon our previous experience a sample size of 15 per treatment group will give 80% power to separate demineralization in the test enamel slabs from the control slabs.

Each sample will exhibit a relative mineral loss value (ΔZ). Means and standard deviations for each group are calculated and the groups are compared statistically by a two-tailed, paired t-test for significance at $P<0.05$.

Updated sample size calculation after performing the pe-study with 5 subjects

After having performed the planned pre-study with 5 subjects, we calculated the sample size for paired differences based on the ΔZ mineral loss results obtained from these 5 subjects. The calculation took into account the mean difference between pairs and the standard deviation of the differences. Based on these data, we assumed that the study would require a sample size of 17 (number of pairs – 17 subjects, each wearing control and Nobio samples) to achieve a power of 80% and a level of significance of 5% (two sided), for detecting a mean of the ΔZ differences of 383 between pairs, assuming the standard deviation of the differences to be ΔZ 507. To account for attrition, we were planning to recruit 20 additional subjects into the study.

Eligibility Criteria for Participants

Inclusion criteria

- aged between 18 and 80 years,
- have at least eight natural teeth remaining and have a recent history of dental caries
- wearing lower partial denture (with replaced teeth on both sides of the mouth)
- willing to wear their denture during the night
- are in good health, of either gender
- are in good current oral health with no active caries or periodontal disease (but with a history of caries)
- have an understanding of the study
- have saliva flow within the normal range (stimulated saliva flow rate of greater than 0.7 ml/minute)
- no antibiotics for the last three months
- willing to comply with all study procedures and protocols,
- residing in San Francisco or other nearby locales with community water fluoridation (to eliminate water fluoridation as a potential confounding variable),
- able to give written consent themselves
- must be able to read and understand English,

- willing to sign the "Authorization for Release of Personal Health Information and Use of Personally Unidentified Study Data for Research" form; data will only be used for research.

Exclusion criteria

- subjects who have less than 8 natural teeth remaining
- subjects who have used a 5,000 ppm fluoride toothpaste in the last 6 months
- subjects who have used Chlorhexidine or other any other antimicrobials (cetylpyridinium etc.) in the last 6 months
- show evidence of extremely poor oral hygiene
- subjects suffering from systemic diseases, significant past or medical history with conditions that may affect oral health or oral flora (i.e. diabetes, HIV, heart conditions that require antibiotic prophylaxis),
- taking medications that may affect the oral flora or salivary flow (e.g. antibiotic use in the past three months, drugs associated with dry mouth / xerostomia),
- other conditions that may decrease the likelihood of adhering to study protocol,
- in-office fluoride treatment within the last three months,
- subjects who will leave the area and are unable to complete the study

IRB approval - ClincalTrials.gov registration

Investigational Review Board approval (UCSF Committee on Human Research - CHR) will be achieved before the study will be started. Since the pilot study is an in situ study it does not need to be registered with ClincalTrials.gov.

Study Duration: anticipated end date June 2019

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