RESEARCH PROTOCOL

I. TITLE Oral Care Intervention in Mechanically Ventilated Adults: Renewal

II. STAFFING

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All personnel will complete required USF Human Subjects training. A comprehensive study training manual will be developed and distributed to all study personnel. The training manual will include a study overview and all data collection procedures and will be updated as needed. Prior to data collection, all study personnel will be trained to perform tasks appropriate to their role in the project by the Project Director including subject recruitment, obtaining consent consistent with applicable state and federal statutes, all interventions and data collection procedures. Role specific training will include intervention procedures, saliva sample collection, and data collection for all personnel. The graduate research assistants (GRAs) who will provide the intervention will be trained on the procedure by the Project Director. After training is complete, each GRA will perform a return demonstration, satisfactorily completing

all critical elements identified (100% accuracy) before interventions will begin. Each GRA will also be tested every 3 months throughout the study period to ensure that all critical elements of each procedure are included. Monthly study meetings will be used to review study procedures and communicate essential information; time sensitive information regarding study operations will be communicated by called study meetings and by e-mail.

III. CONFLICT OF INTEREST

None of the investigators will benefit from subjects' participation in this project or completion of the project in general.

IV. RESOURCES

An R01 continuation proposal has been submitted to the National Institutes of Health to support the conduct of this project. Budget items include personnel costs for investigators and support staff, supplies, and lab analysis for all subjects. Subjects will be recruited at Tampa General Hospital (TGH). Saliva samples will be transported to the University of South Florida (USF) College of Nursing Biobehavioral Laboratory for processing.

V. HYPOTHESIS

Oral care is a standard component of care of the mechanically ventilated patient, and tooth brushing is a customary part of nurse-administered oral care. Despite its prominent position in bedside care, there is little evidence to judge the benefits or associated risks of tooth brushing, and the optimal frequency of tooth brushing in the critically ill has never been experimentally determined. Determining the optimal frequency for tooth brushing in mechanically ventilated adults is an important unresolved issue and will have a significant impact on nursing practice and patient outcomes.

VI. SPECIFIC AIMS

Oral care is a standard component of care of the mechanically ventilated patient, and tooth brushing is a customary part of nurse-administered oral care. Despite its prominent position in bedside care, there is little evidence to judge the benefits or associated risks of tooth brushing, and the optimal frequency of tooth brushing in the critically ill has never been experimentally determined. In addition, the extent to which individual, patient-level factors influence tooth brushing efficacy, mucosal inflammation, and risks is poorly understood. This proposal is the capstone to our long-term goal of providing definitive guidance for effective evidence-based oral care interventions for critically ill adults. In this proposal, we will determine optimal tooth brushing frequency, and use innovative measures of dental plaque and mucosal inflammation while considering individual-level variables that may influence both efficacy and risk of adverse events. The proposed project will complete the essential evidence base for oral care delivered by nurses to critically ill patients.

It is now understood that a nidus of inflammation in the oral cavity might impact systemic health by serving as a trigger for general systemic response detrimental to the critically ill adult. Research on oral care in mechanically ventilated adults has focused on the association between oral health and ventilator-associated pneumonia (VAP). Rates of VAP have fallen dramatically over the past decade as a result of concerted attention to VAP prevention. Current recommendations focus on use of an antiseptic solution to reduce oral microbial colonization and risk of ventilator associated complications; recommendations for routine prescription of chlorhexidine (CHX) are based in part on the results of the first component of this program of research (R01 NR07652)[1-2]. In the initial funding period, we examined the effects of tooth brushing three times a day and CHX twice a day, alone and in combination, on dental plaque and risk of VAP. We found that while CHX reduced risk, tooth brushing neither reduced risk nor enhanced the effect of CHX. In the current funding period, we focused more closely on the role of CHX in reducing VAP risk. We tested the addition of a pre-intubation application of CHX, and surprisingly, found that it did not reduce VAP risk beyond the protection afforded by adherence to post-intubation CHX application guidelines. This is important to clinical practice; since our data indicate that it is not essential to deliver the first dose of CHX prior to intubation, permitting providers to focus their attention on other critical pre-intubation activities.

Despite a lack of demonstrated effect of tooth brushing on VAP prevention in our previous work and other clinical trials to date[2-5], tooth brushing has other potential benefits for mechanically ventilated adults, including control of dental plaque accumulation, maintenance of oral mucosal integrity and reduction of oral inflammation and gingivitis. These benefits may further promote patient comfort and satisfaction. However, these benefits of tooth brushing have not been examined, and there is no evidence in this population to guide optimal frequency of the intervention to maximize benefits. Thus, nurses are left without direction for practice, the contributions of tooth brushing to patient outcomes other

than VAP remain unclear, and great variability in frequency of tooth brushing and in use of nursing time and resources continues to exist.

While potentially beneficial, tooth brushing may have accompanying risks. Our data indicated a worrisome trend toward increased risk of VAP in subjects whose teeth were brushed three times a day.[2] Tooth brushing associated bacteremia has been documented in other populations,[6-11] and microbial colonization from tooth brush contamination is also a theoretical risk.[12] Benefits and risks of tooth brushing may be patient dependent, but little is known about the specific factors that selectively influence outcomes in critically ill adults. Additionally, each episode of tooth brushing uses nursing time, effort, and supplies. Optimizing the frequency of tooth brushing will enable nurses to deliver the best care with the most efficient use of resources.

Because an evidence base for the common intervention of nurse-administered tooth brushing is absent, we propose to complete our examination of oral care interventions in mechanically ventilated adults with a randomized clinical trial of tooth brushing frequency (once, twice, or three times daily) focused on conclusively defining the benefit and risk of varied frequencies, and identification of moderating patient-level factors for risk and benefit. The *project's overall goal of determining optimal tooth brushing frequency for mechanically ventilated adults* will be accomplished through the following specific aims:

1. Evaluate the clinical equivalence (non-inferiority) of three tooth brushing frequencies on oral health (dental plaque and mucosal inflammation) in critically ill adults receiving mechanical ventilation.

2. Quantify and compare the safety of three tooth brushing frequencies on serious adverse outcomes, including ventilator associated events and clinically relevant healthcare-associated infections (HAIs, including VAP, bacteremia, Systemic Inflammatory Response Syndrome and sepsis).

3. Investigate patient factors that influence tooth brushing frequency benefit and risk in critically ill adults (secondary aim).

VII. BACKGROUND AND SIGNIFICANCE

Determining the optimal frequency for tooth brushing in mechanically ventilated adults is an important unresolved issue and will have a significant impact on nursing practice and patient outcomes. Nurses have a high level of enthusiasm for oral care as part of care of the mechanically ventilated patient; our 2004 oral care state of the science paper [13] remains one of the top 10 most-cited papers published by the *American Journal of Critical Care* (http://ajcc.aacnjournals.org/reports/most-cited). However, there has been no published research addressing how frequently tooth brushing should be done to maximize benefits while minimizing risks and optimizing nursing effort. Twice and three times a day frequencies are reported in research and clinical literature, without any evidence for additional benefit or risk related to more or less frequent intervention. Recent consensus guidelines acknowledge that "At present there is no evidence to support an optimal frequency for oral hygiene..." [14]. The proposed project will directly address this gap in the evidence for bedside nursing care, and enable rational selection of intervention frequency.

Tooth brushing is a common nursing intervention which is routinely performed in critically ill adults, and clinical providers hoped that tooth brushing would reduce risk of VAP by reducing microbial burden in the mouth. However, we found that although dental plaque was reduced in subjects who received tooth brushing three times daily, the risk of VAP was not reduced, and in fact trended worse[2]. There is now substantial evidence supporting inclusion of CHX in daily oral care to reduce risk of VAP from our initial findings[2] and confirmed by others in subsequent clinical trials [3-5] and meta-analyses [15]. Additionally, we found that tooth brushing did not enhance VAP protection afforded by CHX [2]. Other RCTs have reported similar findings [4,16,17], undermining the hypothesis of a positive contribution of tooth brushing to VAP risk reduction.

However, tooth brushing has potential benefits which are important despite a lack of involvement in VAP prevention. Because previous tooth brushing research in the ICU has focused primarily on VAP risk reduction, examination of other benefits has been obscured. Tooth brushing reduces mucosal inflammation in healthy populations, and accumulation of dental plaque exacerbates mucosal inflammation. Clinical experimental models of gingivitis frequently use cessation of tooth brushing to initiate gingival inflammation; biomarkers of inflammation in gingival crevicular fluid (GCF) increase in healthy adults within 1 to 3 days of abstaining from tooth brushing [18], continue to rise over at least 21 days [19], and return to baseline as soon as 2 days within resumption of tooth brushing. The effect of tooth brushing on oral inflammation in the critically ill has not been well examined, nor has the systemic effect of oral inflammation been well elucidated. Further, both dental plaque accumulation and mucosal inflammation may be a source

of discomfort, and a nidus of inflammation in the oral cavity might serve to trigger a general systemic response detrimental to critically ill adults [20,21].

While tooth brushing has a high potential for benefit through reduction of dental plaque and subsequent oral inflammation, associated risks in mechanically ventilated adults are not well understood. Tooth brushing associated bacteremia has been documented in other populations [6-8]. Organisms dislodged from dental plaque during tooth brushing might increase risk of HAIs if they are aspirated (contributing to VAP) or translocate into the bloodstream (contributing to bacteremia and sepsis). Microbial colonization from tooth brush contamination is also a theoretical risk. Individual demographic and clinical factors may moderate both benefits and risks of tooth brushing, but these have not been examined in critically ill adults where benefit/risk balance is likely to be different from healthy populations. Additionally, each episode of tooth brushing uses nursing time, effort, and supplies, and displaces other care activities.

The contribution of this proposed research will be a determination of the optimal frequency of tooth brushing in mechanically ventilated adults to maximize oral health benefits while minimizing systemic risks, thus optimizing patient outcomes as well as nursing effort. *This contribution is significant because it will have immediate impact on bedside nursing practice, and is a final component necessary for specific evidence-based guidance for the common nursing intervention of oral care in mechanically ventilated adults*.

We anticipate substantial benefits to be accrued as a result of the proposed study. First, nurses will have evidence to support best clinical practices in the tooth brushing component of oral care. Optimizing the frequency of tooth brushing will enable best use of nursing time and resources, and enable cost containment. Additionally, this project will result in improved understanding of oral health during mechanical ventilation and individual factors that contribute to oral health problems in critically ill adults. Control of dental plaque and reduction of oral inflammation are likely to improve patient comfort, and may reduce systemic sequellae related to a nidus of inflammation in the mouth.

Innovation

This project will be the first to examine the non-VAP associated effects of varying frequencies of a standardized tooth brushing protocol on benefits and risks in mechanically ventilated adults. Nurses determine frequency of tooth brushing in a subjective and individualized manner. Our proposal is innovative in that it will provide head-to-head comparative data for frequency of a common nursing intervention which has not been examined in the context of safety or equivalence. In addition to testing commonly reported tooth brushing frequencies (twice or three times daily), we will also test a once daily protocol to determine whether a minimal frequency can adequately control dental plaque while reducing nursing effort and associated cost.

We also will demonstrate innovation in our evaluations of dental plaque and mucosal inflammation. The proposed project moves beyond the typical subjective clinical assessment of plaque and oral mucosa (such as the commonly used Beck Oral Assessment Scale [22]), and enhances our prior data collection methods. We will enhance the dental plaque scoring system we previously employed (which scores every surface of every tooth) with digital imaging of tooth surfaces for dental plaque scoring. Digital plaque imaging [23,24] facilitates blinding of the dental plaque evaluation and produces archival data which can be better subjected to additional analysis and reliability determinations. We will also include an inflammatory biomarker in GCF for measurement of mucosal inflammation. Recent dental research has identified pro-inflammatory cytokines, and in particular interleukin 1β (IL-1β) as sensitive and robust indicators of oral inflammation in GCF. GCF IL-1β is reflective of the underlying pathophysiological processes related to mucosal inflammation, can be detected before clinically observable changes, and correlates with clinical severity of mucosal inflammation. [19,25,26] Digital plaque images and GCF can be collected noninvasively and are ideal for the prospective serial observations we plan in this proposal. These methods will result in more objective, reliable, and quantifiable measures of oral health. Inclusion of digital plaque imaging and GCF cytokine levels in our measures of oral health adds innovative dimension to our evaluation of optimal tooth brushing frequency.

The data analysis approach for this trial is considered innovative in that it will focus on providing definitive clinical practice guidance through joint evaluation of non-inferiority and quantification of number needed to harm. This parallels how decisions are made in actual clinical practice, as opposed to conventional testing of superiority among respective randomly-assigned treatment regimens. The analytic approach centers on estimation of clinically-relevant parameters that weigh clinical benefit versus harm, thus increasing likelihood of immediate translation of the findings to bedside critical care practice.

In our opinion, this project is highly innovative both in the focus of the intervention and in the methods employed for data collection and analysis.

Summary

Despite its prominent position in bedside care, there is little evidence to judge the benefits or associated risks of nurse-administered tooth brushing for mechanically ventilated adults, and the optimal frequency of tooth brushing in the critically ill has never been experimentally determined. This project will complete the examination of oral care interventions in mechanically ventilated adults with a randomized clinical trial of tooth brushing frequency (once, twice, or three times daily) focused on conclusively defining the benefit and risk of varied frequencies, and identification of moderating patient-level factors for risk and benefit. The project's overall goal is to determine optimal tooth brushing frequency for mechanically ventilated adults. Optimal tooth brushing frequency is important for control of dental plaque and reduction of oral inflammation; it is likely to improve patient comfort, improve efficiency of nursing care, and may reduce systemic sequellae related to oral inflammation. The primary aims of the proposed project are: 1) Evaluate the clinical equivalence (non-inferiority) of three tooth brushing frequencies on oral health (dental plaque and mucosal inflammation) in critically ill adults receiving mechanical ventilation; and 2) Quantify and compare the safety of three tooth brushing frequencies on serious adverse outcomes, including ventilator associated complications and clinically relevant HAIs. A secondary aim is to investigate patient factors that influence tooth brushing frequency benefit and risk in critically ill adults. These objectives will be accomplished using a prospective, randomized, experimental design. Subjects (n=345) will be randomly assigned within 24 hours of intubation to one of three intervention groups which differ in frequency of tooth brushing delivered by study personnel (once, twice, or three times daily). Dental plaque (UM-OHI score, with observations documented and augmented by use of a digital intraoral camera), mucosal inflammation (gingival crevicular fluid IL-1β), and HAIs will be assessed daily during the intervention period. The data analysis will focus on providing definitive clinical practice guidance through joint evaluation of non-inferiority (comparison of dental plaque between groups by analysis of covariance) and quantification of number needed to harm. Repeated measures linear mixed models (treating dental plaque and mucosal inflammation as separate outcome variables) will provide insight as to specific patient-level factors that may modify the clinical effectiveness and safety profile associated with frequency of tooth brushing. Information about efficacy and safety of each frequency of tooth brushing will provide a clear recommendation for optimal tooth brushing frequency with direct translation to clinical practice.

VIII. PRELIMINARY PROGRESS/DATA REPORT

Progress Report

The current project was funded as a continuation of R01 NR007652 9/29/08 through 6/30/12, and is in no cost extension 7/1/12 until 6/30/13. The project focused on evaluating the benefit of adding a pre-intubation CHX dose to the known benefit of post-intubation CHX to reduce the risk of VAP. The primary aim was to test the effect of a pre-intubation oral application of CHX on the development of VAP in a variety of mechanically ventilated, critically ill adults. Secondary aims were 1) to test the effect of a pre-intubation oral application of CHX on early ET colonization in mechanically ventilated adults, and 2) to explore potential biomarkers of VAP development and resolution. The project began at Virginia Commonwealth University (VCU), and was transferred to University of South Florida (USF) 08/01/11, when Dr. Munro accepted a position at USF.

We reasoned that reducing the number of microorganisms in the mouth pre-intubation by application of CHX, added to continual microbial suppression by CHX applied after intubation, would reduce the risk of VAP. This was a logical extension of the work done in the initial funding period, in which we used a randomized controlled 2X2 factorial experimental design to test tooth brushing three times daily and CHX twice daily in newly intubated critically ill adults, and demonstrated that CHX reduced the risk of developing VAP while tooth brushing did not (see preliminary study below).

Enrollment in the current project was initiated at VCU, and subsequent to the transfer of the grant to USF, we opened a second enrollment site at Tampa General Hospital which is associated with USF. A total of 314 subjects were enrolled (VCU, n=214; USF, n=100). Patients with a clinical diagnosis of pneumonia at the time of intubation were excluded from enrollment, as determination of nosocomial pneumonia was confounded in subjects with pre-existing pneumonia. Consent for participation in the research study was obtained prospectively from the subject's legally authorized representative (LAR). Subjects remained in the study for a maximum of 6 days; for subjects who were extubated prior to 6 days, the subject's participation ended on the day of extubation.

Subjects were randomly assigned to 1 of 2 groups; the intervention group (n=157) received oral application of 5 ml CHX gluconate 0.12% solution pre-intubation by swab to the oral cavity administered by study personnel, while the control group (n=157) received no pre-intubation intervention. All subjects (both groups) received CHX twice daily after intubation. Mean age was 58.12 years (16.01). Males comprised 60.19% of the sample; 97.13% were non-Hispanic, 64.33% were white, and 32.17% were black. Enrollment occurred in the pre-operative area for 28.34%, in the emergency department for 27.71%, and the remainder was enrolled in ICUs or during urgent/emergent intubations in other areas of

the hospitals. Airway control was the most frequent indication for intubation (63.06%). Severity of illness score (APACHE III) on admission to the study was 67.13 (SD=29.32), indicating high acuity.

For the primary aim, the effect of a pre-intubation oral application of CHX on the development of VAP, we compared groups using a repeated measures model with Clinical Pulmonary Infection Score (CPIS) [27] as the response variable. Subjects who had complete CPIS data on admission to the study (Day 0) and subsequent complete CPIS data from day 2, 3, 4 or 5 (47 CHX & 47 control, 438 observations) were included in the analysis. There was no statistically significant difference in CPIS scores between the groups over the intervention period. Importantly, mean CPIS in both groups remained below 6 in both groups (Figure 1, mean and standard deviations for each group's CPIS by day), indicating that the risk of VAP was reduced in the presence of CHX even if initiated after intubation. Figure 2 depicts changes from study admission in CPIS for each group by day (better illustrating the lack of significant differences). We conclude that pre-intubation application of CHX did not provide additional benefit in reducing risk of development of VAP when compared to post-intubation CHX.

Figure 1: CPIS Means over Time

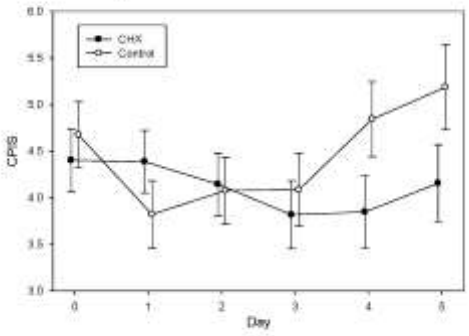
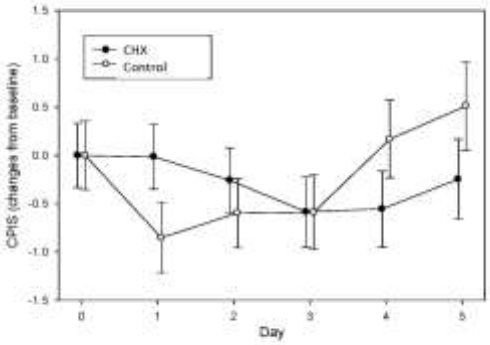


Figure 2. Changes from Baseline in CPIS over Time



In order to test the effect of the pre-intubation oral application of CHX on early ET colonization (secondary aim), a sample was taken by swab from the interior lumen of ETTs in a planned subset of 83 subjects (43 CHX group, 40 control group) from whom the ETT could be obtained at extubation. Potentially pathogenic species were identified by semiquantitative culture, and results were collapsed into 2 categories: colonization (moderate or many organisms) or no colonization. Logistic regression analysis was performed with terms for group, intubation length, and 2 way interaction. A majority of ETTs in both study groups were not colonized at the time of extubation (81.4% CHX, 82.5% control). There was no statistically significant difference in ETT colonization between the groups (p=0.8656). There was a low frequency of ETT colonization at the time of extubation in both the pre-intubation CHX group and the group who received CHX beginning 12 hours after intubation. We conclude that pre-intubation application of CHX did not provide additional benefit in reducing ETT colonization when compared to post-intubation CHX.

We have begun analyses related to potential biomarkers of VAP development and resolution (secondary aim). We collected daily serial blood samples for determination of biomarkers (procalcitonin and a panel of 17 cytokines) in a subset of subjects (CHX n=138 samples; control n=127). All biomarkers were detectable. Nine biomarkers were above the lower limits of detection in more than 90% of samples. Several cytokines (notably, IL-2 and IFNγ), reached detectable values in some samples but were below the lower detection limits of our high-sensitivity assays for a majority of subjects. We reason that our multiplex assays were able to reliably detect these analytes, but that levels of these cytokines were very low in a majority of subjects (below the lower limit of the current most sensitive detection methods). Values were log-transformed for initial correlational analyses. Procalcitonin on study admission was detectable in every sample, and was significantly correlated to pro-inflammatory cytokines, including IL-1β ($p<.0001$) and IL-6 ($p=0.0005$). We are currently undertaking a more detailed examination of the data, including analyses of temporal associations among biomarkers and CPIS, and biomarker patterns over time.

The results of the current project indicate that it is not essential to deliver the first dose of CHX prior to intubation in order to reduce risk of VAP. Importantly, omitting the pre-intubation CHX application simplifies pre-intubation procedures, and enables providers to focus their attention on other critical pre-intubation activities.

Justification and Feasibility

Dental plaque is a significant factor in oral health, with implications for systemic health. Dental plaque is a complex biofilm found on tooth surfaces, and plaque accumulation is enhanced by bacterial aggregation between and among species. Microbial flora are concentrated in dental plaque, which is a complex environmental niche of interdependent microorganisms embedded in bacterial and salivary products.

Accumulation of dental plaque is closely associated with oral inflammation, including gingivitis and periodontitis. The standard clinical experimental model for gingivitis involves with holding of tooth brushing to induce or exacerbate the disease.[18,19,28] In healthy adult subjects who withheld tooth brushing, Scott and colleagues [19] found a progressive, positively correlated increase in dental plaque and GCF biomarkers over the 21 day study period; when inflammation was assessed again after 7 days of brushing resumption, it had resolved. Further, they found that GCF IL-1β increased after 3 days of plaque accumulation, in advance of clinical signs of inflammation. When measured over shorter intervals in a briefer study (tooth brushing withheld for 3 days), Zhang and colleagues found that GCF IL-1β elevated after one day, and returned to baseline within 2 days of resumption of brushing.[18]

Oral mucosal inflammation, and in particular gingivitis or periodontal disease, provides opportunities for bacterial overgrowth, and richly vascularized and inflamed tissues associated with these diseases are susceptible to bacterial invasion. [29] Gingivitis and periodontitis are known to increase the risk of bacteremia related to invasive dental procedures in healthy populations. [30,31] Paradoxically, tooth brushing has also been linked to transient bacteremia in healthy subjects. [32,33] In critically ill adults, central line catheters are the predominant but not the sole source for bacteremia. [34] The role of other sources, including tooth brushing, has not been elucidated.

The mucosal inflammatory response to dental plaque is complex, and in addition to immune stimulation by microbes within dental plaque, the response may also be triggered or exacerbated by non-microbial components of the biofilm. Tooth brushing is widely recognized as the optimal way to remove dental plaque and reduce the associated inflammatory response. Other oral care strategies, such as foam swabs, are not effective in removing dental plaque. [35,36]. Chlorhexidine suppresses microbial growth in dental plaque, but does not remove the physical biofilm.

In the absence of evidence regarding optimal frequency, tooth brushing practices vary. Current guidelines for care of mechanically ventilated adults do not have an evidence based tooth brushing component, and there is not a consensus in the literature regarding appropriate frequency of tooth brushing in the critically ill. The American Dental Association (ADA) recommends that healthy persons brush their teeth twice daily. In preliminary research prior to the R01, we compared usual care to an intervention performed by nurses every 12 hours in critically ill adults (both mechanically ventilated and not ventilated) [37]. In the previous funding period of the R01, tooth brushing was performed three times daily, based on our previous findings of prevalent xerostomia and dental plaque accumulation in mechanically ventilated adults [38]. Three interventions per day is congruent with nursing care patterns of AM, PM, and HS administration for other routine nursing procedures such as back care. Other ICU research protocols, focused on VAP prevention, have included tooth brushing twice daily [39-42] or three times daily [4]. There are no published research reports of once daily tooth brushing in hospitalized patients, and there is considerable resistance among nurses to eliminating it entirely.[43,44]

Surveys of nurses' practices reveal wide discrepancies in clinical practices among institutions and among individual nurses, and a lack of specificity in institutional protocols[45-49]. In the absence of evidence regarding benefit and risk, many nurses have adopted a "more is better" approach to oral care. In our previous research, nurses indicated that they administered oral care as frequently as seven times per day [45], and clinical articles have inappropriately cited our previous publications as support for increased frequency for oral care [50]. Evidence regarding the optimal frequency for tooth brushing is lacking, and is essential to informing nursing practice.

Preliminary Studies

We have substantial experience which enhances the likelihood that we can successfully accomplish the specific aims. We have been active in research related to oral care in critically ill adults for over 2 decades. We have direct and extensive experience in enrolling critically ill subjects; delivering oral care interventions in intensive care settings, including tooth brushing intervention at the maximal frequency proposed; measuring dental plaque and global oral health; obtaining, measuring, and analyzing systemic and oral biomarker data; and quantifying HAIs. In two previous research studies, we quantified and characterized dental plaque accumulation in subjects who were not receiving a standardized oral care intervention. We have successfully completed multiple RCTs, and the data we have generated has had direct impact on clinical practice, including contributing to national guidelines for care of mechanically ventilated adults.

1. Oral Care in Mechanically Ventilated Adults (R01 NR007652, initial funding period). [2] The primary aim of the initial funding period of this R01 was to test the effects of tooth brushing, CHX, and a combination oral care intervention (tooth brushing plus CHX) on dental plaque, oral microbial flora and oral immunity in critically ill mechanically ventilated adults. A secondary aim was to examine the effects of tooth brushing, CHX, and combination (tooth brushing

plus CHX) oral care interventions on development of VAP in critically ill mechanically ventilated adults. A between-subjects 2X2 factorial design was used; 547 subjects were randomly assigned to 1 of 4 treatments: 0.12% solution CHX oral swab twice daily, toothbrushing three times daily, both toothbrushing and CHX, or control (usual care). Oral care interventions were provided by study personnel. Dental plaque was scored on study days 1, 3, 5 and 7 using a standardized scale, the University of Mississippi Oral Hygiene Index (UM-OHI)[51], with observations augmented by use of a dental plaque disclosing agent, visible only under ultra-violet (UV) light so that study personnel providing interventions and clinical providers were not aware of plaque assessment results. Plaque assessors were blinded to subject group assignment. Subjects remained in the study for a maximum of 7 days; participation ended with extubation. Subjects were 61% male, with a mean age of 47.9 years and mean APACHE III score of 77. Serial dental plaque data from admission to the study through day 3 were available for 222 subjects. Mixed model analysis of variance showed a significant effect on dental plaque of the toothbrushing intervention (p=0.0411). The CHX intervention had no effect on the presence of dental plaque (p=0.9397), and there was no interaction effect between toothbrushing and CHX.

In contrast, when we tested the effects of the interventions on VAP risk as measured by the Clinical Pulmonary Infection Score (CPIS), CHX significantly reduced the incidence of pneumonia on day 3 (CPIS ≥6) among patients who had CPIS <6 at baseline (P = .006), while tooth brushing had no effect on CPIS (p = .54) and did not enhance the effect of CHX. We reasoned that although both CHX and tooth brushing reduce organisms in dental plaque, CHX has bacteriocidal activity while tooth brushing mechanically reduces the number of organisms without residual activity on those remaining in the mouth. Thus, dental plaque remained physically present on the teeth of patients who received CHX, but organisms present in the plaque were inhibited. Although tooth brushing physically removed dental plaque, the intermittent reduction in organism numbers achieved by tooth brushing was insufficient to reduce risk of VAP.

This study provided a critical foundation for the proposed project. It provided us with extensive experience in delivering the tooth brushing intervention, conducting dental plaque assessments in mechanically ventilated adults, and interpreting oral health data. It provided important evidence that although tooth brushing 3 times a day did not reduce VAP risk, it did reduce dental plaque. While not statistically significant, the trend toward worse CPIS scores in subjects who received tooth brushing 3 times a day spurred our interest in establishing the optimal frequency for tooth brushing and in testing non-equivalence of less frequent (once daily) tooth brushing intervals.

2. Pilot Study of Oral Care in the Adult Intensive Care Unit. [37]. Prior to the initial funding period of this R01, we conducted a pilot study with primary aims to 1) describe the oral status of critically ill persons hospitalized in a medical respiratory intensive care unit, and 2) evaluate the effects of a defined oral care protocol on the oral health status of critically ill patients. A cohort, quasi-experimental, longitudinal (before/after) study design was used. Sixty critically ill subjects were included in the study, 30 in a control group (whose oral care was performed by the bedside nurse without knowledge of the oral care protocol) and 30 in an intervention group (after nurses had been educated about an oral care protocol). The study did not focus on mechanically ventilated patients, although mechanically ventilated subjects were included.

The oral care protocol included tooth brushing every 12 hours from the day of admission into the study through day 7. Data were collected on the date of admission to the study and on days 3, 5, and 7. Oral assessments, using 100 mm visual analog scales, were completed in the morning on admission to the study and on days 3, 5, and 7 by a dental hygienist and the bedside nurse. The intervention group mean inflammation scores (mean = 3.9 on scale of 0-10, *SEM* = 3.0) were significantly lower ($p = 0.0276$) than the control group (mean = 12.4 on scale of 0-10, *SEM* = 2.2). Although not statistically significant when analyzed by *t*-test, the experimental group also had lower mean values than the control group on a visual analogue scale of plaque (14.2 vs. 18.3). Despite the measurement by visual analogue scale being less robust than methods in our subsequent studies, this very early study provided us with initial data suggesting that tooth brushing in critically ill patients could affect both mucosal inflammation and dental plaque.

3. Backrest Position and Oral Health: Effect on VAP (R15 NR04730, PI: Grap, Co-I: Munro). This non-experimental, longitudinal study of 66 mechanically ventilated adults was conducted to describe 1) the relationship of backrest elevation on the development of VAP, and 2) the relationship of oral health status on the development of VAP. Assessment of oral health status on study days 1, 4, and 7 included biomarker measurement (salivary lactoferrin and IgA), DMFT, a visual analogue scale of dental plaque, and semi-quantitative oral and endotracheal aspirate cultures. Data were analyzed using a mixed linear model.

The analysis of specific aim 2 revealed complex relationships among severity of illness, dental plaque scores at baseline and on day 4, and CPIS at baseline and on day 4. Correlations were significant with day 4 CPIS for APACHE II (P=.007), day 4 salivary volume (P=.02), interaction of APACHE II score and day 1 CPIS (P<.001), and interaction of day 1 CPIS and plaque (P=.01). The model indicated that increased dental plaque was most predictive of pneumonia in patients with high APACHE scores and lower baseline CPIS. The number of organisms present in oral cultures increased

from Day 1 to Day 4, and remained high on Day 7. In addition to identification of non-pathogenic oral flora, we were successful in identifying potential VAP pathogens in oral cultures (including *S. aureus, P.aeruginosa,* and *A. baumanii*) preceding or concurrent with their appearance in endotracheal aspirates. These results indicated that oral health of critically ill patients was often compromised at the time of intubation, and deteriorated over time. Additionally, this study provided our first opportunities to collect salivary and microbiological samples from critically ill adults, and to analyze salivary biomarkers.

4. Dental Plaque Accumulation in Mechanically Ventilated Adults.[52] In a sub-study of this R01 during its initial funding period, dental plaque data was evaluated longitudinally in a subset of the control group enrolled within 24 hours of intubation in the first year of data collection of R01 NR007652 (n=137). Subjects in the control group received usual oral care delivered by the bedside nurse; nurses were free to individualize the method of oral care, and the study units did not have protocols that specified tools and solutions for oral care. Baseline oral health status was assessed by a count of decayed, missing, and filled teeth (DMFT). Dental plaque was assessed using the UM-OHI [51] on study days 1, 3, 5 and 7; observations were augmented by use of a dental plaque disclosing agent, visible only under ultra-violet (UV) light. This dental plaque assessment strategy was substantially more valid and reliable than our previous VAS. On the day of intubation, subjects had greater than 73% mean dental plaque coverage of tooth surfaces, and mean dental plaque remained greater than 60% through day 7, with statistical differences only between day 1 and day 7 (p = .03). We further examined the pattern of dental plaque accumulation by location (buccal or lingual) and by tooth type (molars, premolars, canines, incisors). Dental plaque averages for the buccal (67%) and lingual (68%) surfaces were equivalent. The molars and premolar tooth types had the most dental plaque (greater than 70%) when compared to the other tooth types. These data reinforced earlier preliminary results regarding preexisting oral problems in mechanically ventilated adults, and further quantified high levels of dental plaque and DMFT scores. Further, the data indicated that usual oral care provided by bedside nurses was not effective in plaque removal.

5. Comparison of biomarkers in blood and saliva. [53] The objective of this study, funded by the current project and a VCU HHMI Science Education Project in Systems Biology Summer Scholar Award (to S.Williamson), was to compare multiple biomarkers (27 cytokines) in plasma samples, passive drool saliva samples, and filter paper saliva samples in 50 healthy adults. Demographic data and three samples were obtained from each subject: saliva collected on filter paper over 1 minute, saliva collected by passive drool over 30 seconds, and venous blood (3 mL) collected by venipuncture. The 27 cytokines selected were bundled together in the commercially available cytokine multiplex assay, reflect both pro-and anti-inflammatory immune response, and are commonly used as a panel in assessment of immune function. Assays were conducted using Bio-Rad multiplex suspension array technology. Descriptive statistics and pairwise correlations were used for data analysis. The sample was 52% male and 74% white. Mean age was 26 (range= 9–63 years, sd = 9.7). The most consistent and highest correlations were between the passive drool and filter paper saliva samples. Between passive drool and filter paper saliva samples, statistically significant correlations were found among 16 of the 27 tested cytokines, including IL-1β, IL-1ra, IL-4, IL- 7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, G-CSF, IFN-γ, IP-10, MCP-1, MIP-1β, and VEGF. Because the oral cavity represents a distinct environmental niche, and immune biomarkers are influenced by processes of local immunity, we predicted that salivary values would differ from plasma values, and we were correct. Between plasma and passive drool saliva samples, only 3 cytokines were statistically significantly correlated (IL-6, IFN-γ, and MIP-1β). This small descriptive correlational study in healthy adults is important because it demonstrates our ability to use a filter paper method for collection and analysis of oral biomarkers, as we will do with GCF IL-1β in the proposed project.

6. Risk Reduction: Oral Care, Bacteremia, and Critical Care.[34,54] In a companion project to the initial R01 funding period (F31 NR009596, Fellow: D. Jones), the goals were to determine (1) the incidence of transient bacteremia related to tooth brushing in mechanically ventilated critically ill adults; (2) the relationship of oral microbial cultures and dental plaque scores to the incidence of transient bacteremia, clinical outcomes, and indicators of infection; and (3) the relationships among patient characteristics and clinical outcomes. All subjects (n=30) received a tooth brushing intervention twice daily for up to 48 hours. Three blood samples (before tooth brushing, 1 minute after the tooth brushing intervention, and 30 minutes after the intervention) for quantitative culture (lysis filtration) were collected at the first and last scheduled interventions. Five subjects (17%) had positive oral cultures for organisms other than normal oral flora (including *S. pneumoniae, coagulase-negative Staphylococcus, E. coli, S. aureus*, and *P. aeruginosa)* before the first tooth brushing intervention. None of the 30 subjects had evidence of transient bacteremia by positive quantitative blood cultures before or after the tooth brushing interventions. Patient characteristics were not statistically significant predictors for systemic inflammatory response syndrome, length of hospital stay, or length of intubation. In this small sample, dental plaque, DMFT and APACHE score were not found by logistic regression to be independent predictors of SIRS (likelihood ratio chi-square = .79, P = .85). Despite the small sample size and limited length of data collection, this project provided

the opportunity to explore tooth brushing risk factors which we will further investigate in the proposed larger project, with a longer planned intervention period and post-extubation follow up.

Taken together, the preliminary studies presented in this application provide a substantial foundation for the proposed work, and clearly point to the examination of the frequency of tooth brushing as the final critical step in developing the evidence base for oral care in mechanically ventilated patients. Further, the preliminary studies document our expertise in the content relevant to and methodologies of the proposed project.

IX. RESEARCH DESIGN AND METHODS

Design

We will use a prospective, randomized, experimental design to accomplish the specific aims. Subjects (n=345) will be randomly assigned within 36 hours of intubation to one of three intervention groups which differ in frequency of tooth brushing (once, twice, or three times daily). The intervention, a standardized tooth brushing protocol developed and tested in the previous project, will be delivered by study personnel. Subjects will continue to receive scheduled interventions for the first 7 days of intubation, or until extubated if extubation occurs within 7 days; for subjects extubated within the intervention period, data collection will continue on days 1, 3, and 5 post-extubation. Dental plaque assessment, GCF for IL-1β determination, and data regarding HAIs and clinical status (for example, severity of illness) will be collected daily by study personnel who are blinded to group assignment.

All subjects will receive standard clinical care for mechanically ventilated patients, as per clinical guidelines and clinical agency guidelines. Standard clinical care includes all components of the IHI ventilator care bundle,[1] including daily CHX. All subjects will receive oral comfort care by clinical providers at the provider's discretion. We will monitor reported compliance with the ventilator care bundle, and record characteristics and frequency of oral comfort care provided in order to assure that groups do not differ in usual clinical care.

Sample. The sample of 345 subjects will be drawn from all patients over the age of 18 years admitted to the Intensive Care Units at TGH who have been intubated less than 36 hours and are being mechanically ventilated. Both male and female subjects from all ethnic and racial backgrounds will be recruited. Subjects will be randomized to group according to a permuted block design developed by Dr. Kip such that after every *k*
subjects balance will be maintained between the groups. For inclusion, subjects must be within 36 hours of initial intubation, have at least one tooth (to permit plaque assessment and GCF collection), and they or their legally authorized representative (LAR) must be able to provide informed consent in English or Spanish. Exclusion criteria include anticipation by the clinical provider of imminent patient death, or medical contraindication to tooth brushing (for example, unstable facial fractures). In order to fully enroll the study during the requested 4 year funding period, we will target enrollment of 10 subjects per month; in the current project, we exceeded this monthly target in our TGH sample.

Setting. Subject recruitment, enrollment, and interventions will be conducted at Tampa General Hospital (TGH) in Tampa, Florida. As one of the largest hospitals in Florida, TGH is licensed for 1,004 beds. Approximately 73.6% of admissions are white, 20.7% are African-American, and 17.2% are Hispanic. TGH is the area's only level I trauma center and one of just four burn centers in Florida. In the current project, we have established excellent research relationships and been successful in meeting target enrollments. Lucia Hamilton (Project Director) is credentialed by the TGH Office of Clinical Research.

Method

Intervention. The tooth brushing intervention was developed in conjunction with dental hygienist researchers and consistent with American Dental Hygienist Association recommendations. It has been deployed successfully in our previous projects, including the large RCT conducted as the initial project of R01 NR007652 (see Preliminary study 1), and is described fully in the publication of the project's primary findings. [2] In the initial project, 229 subjects were randomly assigned to tooth brushing, and the intervention was provided three times daily. In the proposed project, subjects will be randomized to receive tooth brushing once, twice, or three times a day.

In brief, we will systematically brush all tooth surfaces, gums, and tongue with a soft pediatric toothbrush, using dry mouth toothpaste, for two minutes per intervention; length of the intervention will be measured using a timer. The tooth surfaces will be brushed using a gentle vibrating, back and forth, rolling motion, beginning with buccal surfaces, proceeding to lingual surfaces, and concluding with biting surfaces, for 5 strokes on each tooth surface. In areas of the mouth where teeth are missing, the intervention provider will brush the gums. Then, the tongue will be brushed. If the subject is unresponsive and/or has clenched the mouth shut, we will use a mouth prop to gently open the mouth. Following tooth brushing, the mouth will be rinsed with alcohol-free mouthwash and oral fluids will be removed by suctioning the

mouth with an oral suction tip. Moisturizing gel will be gently applied to the oral mucus membranes. The complete intervention requires approximately 15 minutes.

We have found that soft pediatric toothbrushes are more easily maneuverable in the mouths of endotracheally intubated subjects and enables removal of plaque without disturbing oral tubes. Soft bristles reduce potential for trauma and bleeding, and in our previous studies were not associated with adverse effects such as damage to mucosa which can result from hard bristled toothbrushes. We considered use of an electric toothbrush, but selected the manual toothbrush for two primary reasons. First, electric toothbrushes have been associated with significantly increased risk of transient bacteremia compared to manual toothbrushes in healthy populations.[6,10] Second, electric toothbrushes have been tested in two VAP trials, and outcomes were no better than trials in which manual toothbrushes were used (neither manual nor electric toothbrushes have been shown to reduce VAP risk). [16,17] Furthermore, the additional cost of an electric toothbrush as a standard of care will hinder broad adoption of the study findings to clinical practice settings.

We have also chosen not to use disposable toothbrushes. We reason that requirement for a disposable toothbrush will reduce translation to practice related to increased cost. Further, we recently examined environmental contamination of toothbrushes in the ICU (F31NR012340, Fellow: M. Frazelle). [12] While 14 of 100 toothbrushes became contaminated with potential pathogenic organisms over 24 to 72 hours of use, there was a significant association between tooth brush contamination and the use of a storage container (p-value = 0.01), regardless of whether the toothbrush was reused for 24, 48, or 72 hours. Thus, our intervention will include rinsing the toothbrush well after use, and storage in a dry well ventilated space apart from other patient care items (a condition in which none of the toothbrushes studied became contaminated).

Key Variables and Measurement

In this final phase of our investigation of oral care in mechanically ventilated adults, our goal is to determine optimal tooth brushing frequency. Taken as a whole, the work conducted in R01 NR007652 (the proposed project combined with the results of the previous and current projects) results in clear, complete, evidence-based guidance for nurses providing oral care to mechanically ventilated adults.

The primary aims of the proposed project are: 1) Evaluate the clinical equivalence (non-inferiority) of three tooth brushing frequencies on oral health (dental plaque and mucosal inflammation) in critically ill adults receiving mechanical ventilation; and 2) Quantify and compare the safety of three tooth brushing frequencies on serious adverse outcomes, including ventilator associated complications and clinically relevant HAIs (including VAP, bacteremia, and sepsis). A secondary aim is to investigate patient factors that influence tooth brushing frequency benefit and risk in critically ill adults.

We will accomplish these objectives using a prospective, randomized, experimental design. Subjects (n=345) will be randomly assigned within 36 hours of intubation to one of three intervention groups which differ in frequency of tooth brushing (once, twice, or three times daily). The intervention, a standardized tooth brushing protocol developed and tested in the previous project, will be delivered by study personnel for 7 days, or until extubated (if extubation occurs within the first 7 days). Key variables and their measurement intervals are presented in Table 1, and will be described more fully in the Research Design section.

Table 1: Key variables

Concept	Variable	Measurement interval
Oral Health (Specific Aim 1)	Dental Plaque: UM-OHI, augmented with DPIA	Daily for 7 days while intubated, days 1,3,5 post-extubation*
	Gingival inflammation: GCF IL-1β	Daily for 7 days while intubated, days 1,3,5 post-extubation*
Serious outcomes (Specific Aim 2)	Systemic Inflammatory Response Syndrome criteria	Daily for 7 days while intubated, days 1,3,5 post-extubation*
	HAI: positive epidemiologic surveillance report for VAE (including VAP), bacteremia, sepsis	Daily for 7 days while intubated, days 1,3,5 post-extubation*
	Length of stay (ICU and hospital)	Calculated at hospital discharge
	Length of intubation	Calculated at time of extubation
Patient factors (Specific Aim 3)	Demographics (age, gender, smoking status)	On study admission
	Severity of Illness: APACHE	On study admission
	Global oral health: DMFT	On study admission

*Post-extubation data will not be collected on subjects who remain intubated beyond the 7 day intervention period.

Oral Health: Dental Plaque. Dental plaque will be assessed using the University of Mississippi Oral Hygiene Index (UM-OHI),[51] with observations documented and augmented by use of a digital intraoral camera (obtaining images of every tooth at each observation for later analysis). The UM-OHI assessed prior to the first tooth brushing intervention, and daily every day for the first seven days of intubation. If extubated during the 7 day intervention period of the study, the UM-OHI will also be assessed following extubation on days 1,3 and 5 post-extubation to document resolution or persistence of dental plaque after resumption of self-administered oral hygiene. We will use dental hygienists to train study personnel to obtain the digital images, and as plaque assessors.

The UM-OHI assesses every tooth, and divides each tooth into more sections than standard tools used in dental practice, thus enhancing the ability to quantify plaque. The UM-OHI divides the oral cavity into 12 regions: left and right posterior teeth and anterior teeth in each arch, further subdivided into buccal (cheek side) and lingual (tongue side). Each tooth is divided into 5 sections for the buccal surface and 5 sections for the lingual surface. The 5 sections include the mesial, distal and middle sections (which is further subdivided horizontally into gingival, middle and occlusal sections). Each section (total of 10 for each tooth) is scored for the presence or absence of plaque. If plaque definitely exists in the section, it is scored a 1; if no plaque is present, the section receives a 0. Thus, each tooth is scored from 0 (no plaque) to 10 (plaque in every section). The mean plaque score for the subject is then calculated by dividing the total score by number of teeth.

In the initial project, plaque assessors who were blinded to group assignment visually scored dental plaque using the UM-OHI in real time at the bedside. In the proposed project, we will obtain digital images of all teeth (buccal and lingual) using an intraoral camera, and plaque assessors will visually score blinded images downloaded to a computer in the research office at a later date. Digital Plaque Image Analysis (DPIA) has been used to assess dental plaque in studies of dentifrices and other oral hygiene products. Bellamy and colleagues [24,56,57] developed a DPIA system which captures images of healthy subjects' teeth via an external digital computer-controlled camera under white light without disclosing agents, as well as image processing and image analysis software that identifies color differences indicative of dental plaque. DPIA systems used in dental research (and designed for healthy subjects) will not serve our purpose, because they require a cooperative subject, sitting upright, and positioned in a cephalometric head restraint apparatus in order to obtain external images. However, intraoral cameras are now available, and these small portable handheld units (appearing similar to a digital oral thermometer with a camera positioned at the tip of the probe) are able to capture images of buccal and lingual tooth surfaces in white light outside a dental laboratory setting.[23].

While the plaque assessment procedures used in our previous project worked sufficiently, we believe that documenting the condition of the teeth using an intraoral camera, in conjunction with plaque assessors applying UM-OHI assessment to those images, will provide several important advantages. Digital images of subjects' teeth provide documented, archival raw data which can be scored by multiple plaque assessors as needed to enhance both reliability and inter-rater reliability. Positioning the camera in the oral cavity will provide better visualization of the lingual surfaces of teeth, particularly teeth in proximity of the endotracheal tube, than direct external observation; this will enhance detection of plaque on surfaces which are difficult to directly observe. Intraoral imaging can be done more quickly than full bedside assessment, reducing any subject discomfort during the assessment. Conducting the UM-OHI scoring in the research office rather than at the bedside enhances blinding of the assessment. These procedures will also make the most efficient use of plaque assessor expertise.

Oral Health: Oral Mucosal Inflammation. We will quantify mucosal inflammation by determination of GCF IL-1β. Collection GCF is a well-developed dental research methodology dating to the 1960s,[58] and has elements common to our expertise in collection of saliva and quantification of salivary biomarkers. GCF samples will be obtained prior to the first tooth brushing intervention, and daily every day for the first seven days of intubation. If extubated during the 7 day intervention period of the study, GCF samples will also be obtained on days 1,3 and 5 post-extubation to document resolution or persistence of inflammation after resumption of self-administered oral hygiene. Periopaper strips will be placed into the gingival margins of the maxillary premolars for thirty seconds; if these teeth are not present, the next adjacent teeth will be used. Using published methods [25,28], samples will be placed in PBS-BSA transport medium and frozen at -70oC until assay, when the GCF analytes will be eluted from the paper strips and assayed in duplicate for IL-1β using a commercial enzyme-linked immunosorbent assay (ELISA) system according to the manufacturer's instructions.

Serious Outcomes: SIRS, VAE and HAIs. We will obtain data from the subject's medical record to evaluate SIRS criteria (heart rate > 90, respiratory rate > 20 or PaCO2 < 32 mm Hg, WBC > 12,000 mm3 or < 4000 mm3 or > 10% bands, temperature > 38 _C or < 36 _C) prior to the first tooth brushing intervention and daily thereafter while tooth brushing interventions continue. We will also monitor the results of any blood cultures obtained for clinical purposes from our subjects during the study period in order to identify clinical bacteremia. TGH has a robust surveillance program for

identification of bacteremia, VAC (including VAP), and sepsis. We will obtain HAI surveillance data regarding all study subjects from the Hospital Epidemiologist, as well as collecting data from each subject's medical record regarding diagnoses of these conditions by the clinical providers. We will also calculate length of intubation and length of stay for all subjects.

Sample Description and Patient Factors. Data will be collected from the medical record on additional factors in order to provide a comprehensive description of the study sample and conduct an exploration of the influence of individual-level factors on tooth brushing benefit and risk. Demographic data will be collected on study admission, and include age, gender, race, ethnicity, and smoking status. Preexisting global oral health will be measured by DMFT (Decayed, Missing, and Filled Tooth) Index [59] assessed at study admission. The DMFT is a classic instrument which is widely used in dental practice and research. The score is calculated by visually inspecting each tooth and adding the number of decayed teeth, absent teeth, and filled teeth. Scores can range from a score of 0 (all teeth present and without caries) to a score of 32 (every tooth evidencing dental disease through loss, active dental caries, or repaired dental disease). The summative score will be used in examination of the exploratory aim. Severity of illness will be documented on admission to the study using the Acute Physiology, Age, and Chronic Health Evaluation (APACHE III).[60] The APACHE III total score may range from 0 to 299 and consists of the following sub-scores: vital sign/lab, pH/pCO2, neurological, age, and chronic health. Scoring is done using the worst values for the first ICU day.
The APACHE III will be used to describe the study population, to document the initial level of illness severity between the intervention groups, and explore the individual-level variables that may affect tooth brushing benefit and risk. Additional data to describe the sample will include: reason for admission to the unit; administration of antibiotics prior to entry into the study; type of intubation (emergent, oral vs. nasal); daily ventilatory support data (ventilator mode, rate, type of support, positive end-expiratory pressure, pressure support ventilation, FiO_2, PaO_2, and SaO_2) and daily enteral nutrition data (presence, route, rate, type of enteral nutrition, and highest daily gastric pH); and presence of antibiotic, histamine blocker, proton pump inhibitors, antacid, and vasopressor therapy during the study period.

Procedures

Personnel training. Before subjects are enrolled in the study, a comprehensive study manual will be developed and distributed to all study personnel describing all study procedures. Under the supervision of the Principal Investigator, the Project Director will train the GRAs on all procedures. Training will include completion of the USF investigator/researcher educational program for human subject protections, Collaborative Investigator Training Initiative (CITI), developed by experts in the field of human subject's protections. The study staff will be trained on all aspects of data collection, using all the monitoring equipment, as well as the medical record. Training on the entire procedure will be conducted by the Project Director and GRAs have achieved 100% accuracy of all procedures and data collection processes including subject recruitment, obtaining consent consistent with applicable state and federal statutes, intervention procedures, saliva sample collection, and taking digital images. After training is complete, each GRA will perform return demonstrations on all study procedures with the Project Director satisfactorily completing all critical elements identified (100% accuracy) before conducting study procedures independently. Each GRA will also be tested every 3 months throughout the study period to ensure that all critical elements of the procedure are included. This testing will be done by direct observation of study personnel conducting study procedures by the Project Director and will include reliability testing for data collection processes. If any critical element is found to be omitted or inaccurate, retraining and return demonstrations will occur immediately until the omissions are corrected. If study procedures are in progress, the Project Director will supervise study procedures to ensure that all critical elements are present until retraining and revalidation of the GRA can occur. Once data collection begins, the Project Director will review all data for completeness and appropriate entries before the data are entered into the study database. At the beginning of the data entry process a minimum of 1 in every 10 participant records will be checked against the original medical record for data errors. Records for review will be randomly chosen by the data manager. As the study progresses, the frequency of monitoring will be based on the results of previous data review. If the error rate is unacceptable then the monitoring will increase until the error rate is acceptable (less than 5% of all data).

Non-patient participants. Approximately 10 non-patient participants will be invited to provide saliva samples to validate the GCF collection process. Approximately 5 non-patients will be invited to provide a video of their tooth surfaces before and after tooth brushing to validate the randomized digital image selection method for plaque analysis. Each non-patient participant will be consented to publish the analysis results of the GCF samples or tooth surface videos. Non-patient inclusion criteria: must be =/> 18 years of age; have at least one tooth to obtain saliva sample for GCF analysis or tooth surface video; and understand English for informed consent to publish analysis results of GCF sample or tooth surface video. Non-patient exclusion criteria: <18 years old or edentulous and cannot obtain saliva for GCF

analysis or video of tooth surfaces for plaque analysis. Non-patient recruitment procedure: the Project Director will verbally announce availability to participate along with the eligibility criteria to the study team members and to the faculty and staff members of the USF CON Biobehavioral Lab; the Project Director will confirm each volunteer is =/> 18 years of age; has at least one tooth; and understands English; the Project Director will explain the consent form in detail and assess participants level of understanding by requesting a return explanation of the consent to publish the analysis results of the GCF sample or tooth surface videos.

Recruitment and enrollment. All patients admitted to the Intensive Care Units at TGH who are intubated will be assessed for possible inclusion in the study. The PI, co-investigators, Project Director or GRAs will make daily rounds to the adult ICUs and ask the charge nurse to identify any patients, by room number, that have been intubated within the last 36 hours. A HIPAA waiver for screening and recruitment is requested for this study to allow the study staff to review the medical records for screening and recruitment purposes. If a patient meets study criteria (age 18 or older, within 36 hours of initial intubation, have at least one tooth, and they or their legally authorized representative able to provide informed consent in English or Spanish) and has no exclusions (anticipation by the clinical provider of imminent patient death, or medical contraindication to tooth brushing), the potential subject will be approached for a discussion about willingness to participate. If the subject is unable to provide consent due to sedation or inability to communicate, the legally authorized representative (LAR) will be approached, the study will be reviewed and permission for the subject to participate will be obtained from the LAR; in these cases, we will attempt to obtain assent of the subject as well, and will make additional attempts to consent the subject directly as their condition improves. In our experience, potential subjects who are critically ill, especially during the first 36 hours of intubation, are always sedated to some degree, and their ability to communicate effectively is compromised by endotracheal intubation; for these reasons, permission for the subject's participation has generally been obtained from the LAR. However, there may be rare occurrences when potential subjects are adequately oriented and lucid to be able to provide informed consent. Therefore, we will use a 2 step procedure (developed by Fan et al[61]) to determine the patient's ability to provide informed consent. Step 1 is objective evaluation with the Richmond Agitation-Sedation Scale (RASS) and the Confusion Assessment Method for Intensive Care Unit (CAM-ICU). Patients who score -1, 0, +1 on the RASS indicating drowsy, alert, or restless respectively and are not delirious (CAM-ICU = No) will move to Step 2. Step 2 is assessment for competency using the MacArthur Competence Assessment Tool for Clinical Research: MacCAT-CR. This is the most widely used tool, and is the only tool that evaluates all four capacity domains (understanding, appreciation, reasoning, expression of a choice). Evaluation of Step 1 requires approximately 2-3 minutes, Step 2 requires approximately 15 minutes. Fan et al. found in consent evaluations of 150 ICU patients that 89% were sedated/delirious, and unable to provide consent; our experience in previous research is similar. Therefore, we expect that few potential subjects will move to Step 2. The LAR will then be contacted for those who fail Step 1 or Step 2. Subject's willingness to continue participation will be assessed on an ongoing basis throughout the project. We will follow the USF and TGH IRB Written Policies and Procedures, as well as the Florida regulations regarding appropriate use and definition of the LAR. The subject and the subject's LAR will be provided with an oral explanation of the nature of the study and study information, and information in writing, prior to seeking consent signatures. The information will include all elements required for informed consent, and will include all pertinent contact information as well as information about withdrawal from the study. Written documentation of consent will be obtained from the subject, if capable, or the LAR. If the potential subject or LAR does not wish to participate in the study, no additional interactions with the potential subject will take place. Further, subject's willingness to continue participation will be assessed on an ongoing basis throughout the project.

Once the subject has been enrolled, the Project Director will assign the subject to one of the three groups using a randomization schedule generated by Dr. Kip. A set of sequenced, sealed opaque envelopes will be prepared, each containing the assignment of an individual subject. At the time of enrollment, the Project Director will open the next envelope and proceed accordingly. Dr. Kip and the Data Manager will each keep a master copy of the randomization assignment.

Intervention and data collection procedures. Following obtainment of informed consent from the subject or LAR, subjects will be randomly assigned within 36 hours of intubation to one of three intervention groups which differ in frequency of tooth brushing (once, twice, or three times daily). The intervention, a standardized tooth brushing protocol developed and tested in the previous project, will be delivered by study personnel for 7 days, or until the subject is extubated if extubation occurs within the first 7 days.

Daily saliva periopaper GCF samples will be collected and digital images of teeth will be taken and identified only by the subject's arbitrary study number, not by any identifiable private information. GCF samples will be frozen at -70 degrees in the Tampa General Hospital Office of Clinical Research Laboratory, and later transported to the

Biobehavioral Laboratory at the USF CON, and stored securely in the locked, limited access laboratory. Digital images of teeth will be obtained at close range such that photos will not include subject facial features.

Data will be obtained from the patient's medical record, from assessment of the subject's mouth, and from biologic fluids (gingival crevicular fluid, GCF, collected on filter paper). On study admission, the following data will be collected: demographic data (age, gender, smoking status, reason for admission), severity of illness data (APACHE information), and a count of decayed, missing and filled teeth (DMFT). The following data will be collected every day through the first seven days of mechanical ventilation, or until extubated if extubation occurs within the first seven days: digital photos of all teeth (for use in UM-OHI dental plaque assessment), a sample of GCF collected on Periopaper (for GCF IL-1β assay), Systemic Inflammatory Response Syndrome criteria from the medical record, positive epidemiologic surveillance reports for enrolled subjects for VAE (including VAP), bacteremia, and sepsis from the TGH Epidemiology Department records. For subjects who are extubated during the first 7 days of mechanical ventilation, the same data collected during the study intervention period will also be collected after extubation on days 1,3,5 after extubation. Data regarding length of intubation, length of ICU stay and length of hospital stay will also be calculated from the medical record.

All data, including digital photos, will be collected on laptops in the clinical setting; these laptops will remain in the possession of study personnel at all times (unless locked in a cabinet in the locked research office), and all study-related files will be password protected. At the conclusion of each subject's participation, that subject's data will be transferred from the laptop computer to a secure, password protected file on the USF Health server, under the direct supervision of Dr. Kip , with access limited to study personnel. Study information obtained will be kept strictly confidential, and it will not be possible to identify any participant from the reports that may result from this research.

X. PLAN FOR CONTROL OF INVESTIGATIONAL DRUGS, DEVICES, AND BIOLOGICS

N/A

XI. DATA ANALYSIS PLAN

The data analysis approach for this trial is considered innovative in that it will focus on providing appropriate clinical practice guidelines through testing of non-inferiority and quantification of number needed to harm, as opposed to conventional testing of superiority among respective randomly-assigned treatment regimens. Specifically, we will first evaluate whether the single tooth brushing regimen is clinically equivalent (non-inferior) to brushing two or 3 times daily, and also quantify the respective risk versus clinical benefit of brushing multiple times daily. The sample size and power analyses (see below) are based principally on specific aim #1 and a one-sided test of non-inferiority for the once daily tooth brushing regimen.

Specific Aim 1 is to evaluate the clinical equivalence (non-inferiority) of three tooth brushing frequencies on oral health (dental plaque and mucosal inflammation) in critically ill adults receiving mechanical ventilation. To evaluate non-inferiority, we will use data from subjects who have at least 2 days duration of mechanical ventilation and at least 2 days of their randomly assigned tooth brushing regimen. Recognizing variable length of hospital stay and intubation, we will determine as the primary outcome the maximum difference in dental plaque since baseline assessment at the time of intubation. Whereas we would expect balanced distribution of the specific post-admission day in which this maximum difference is achieved between the 3 treatment arms (e.g. day 4, day 5, etc.), timing of the assessment will be included as a covariate along with baseline dental plaque score. Analysis of covariance (ANCOVA) will be used to compare adjusted dental plaque scores between the 1 and 3 times daily tooth brushing regimens. The test for non-inferiority of the single daily brushing regimen will be based on the 95% confidence interval for the adjusted difference between the 2 groups. For this analysis, we define non-inferiority as a modest between-group effect size of 0.50 or less (i.e. modest to low treatment effect associated with tooth brushing 3 times daily). Thus, if the upper limit of the confidence interval for the effect size is <0.50, then the single daily brushing regimen will be considered non-inferior to the 3 times daily tooth brushing regimen. If the non-inferiority test is not supported, a secondary test of the 1 versus 2 times daily brushing regimens will be conducted. Using this strategy, a direct test of non-inferiority of 2 versus 3 times daily is not required (see Table 2).

Specific Aim 2 is to quantify and compare the safety of three tooth brushing frequencies on serious adverse outcomes, including ventilator associated complications and clinically relevant healthcare-associated infections. For aim #2, a composite HAI endpoint will be constructed consisting of VAP, bacteremia, and sepsis. Since HAIs are anticipated to be rare events across the 3 tooth brushing regimens, no formal between-group statistical testing will be conducted. Instead, 95% confidence intervals will be calculated for the proportion of subjects with HAI in each treatment arm. In addition, using person days of observation (i.e. days since intubation), we will calculate incidence rates (IR) of HAI by treatment arm and then calculate the number needed to harm (NNH) using the single daily brushing regimen as the

reference group and the formulas: $NNH_{21} = (1 / (IR_2 – IR_1))$ and $NNH_{31} = (1 / (IR_3 – IR_1))$ where IR_n = observed incidence rate in each treatment arm. In the event that the formal test of non-inferiority fails for the single daily brushing regimen, we will define clinically-relevant "compromised" safety as an NNH of 100 per less. This would be interpreted as 100 individuals or less being treated with the multiple daily tooth brushing to develop an HAI that otherwise would not have occurred with the single daily brushing regimen.

Clinical recommendations defined *a priori* and developed from the analyses conducted for aims 1 and 2 will be developed as defined in Table 2.

Table 2. Application of study findings to tooth brushing frequency recommendations

Comparison Groups[a]	Non-Inferiority	NNH	Recommendation	Rationale
1 versus 3	Supported	N/A	1 time/day	1 time/day non-inferior and safe
1 versus 3	Not supported	≥100	3 times/day	1 time/day inferior, 3 times/day safe
1 versus 3	Not supported	< 50	1 time/day	1 time/day inferior, but 3 times/day safety concern
1 versus 3	Not supported	50 to 100	See 1 vs. 2	1 time/day inferior, but 3 times/day safety concern
1 versus 2[b]	Supported	N/A	1 time/day	1 time/day non-inferior and safe
1 versus 2[b]	Not supported	≥100	2 times/day	1 time/day inferior, 2 times/day safe
1 versus 2[b]	Not supported	< 100	1 times/day	1 time/day inferior, but 2 times/day safety concern

[a]Times per day of tooth brushing. [b]Conducted if the 1 versus 3 test of non-inferiority is not supported and NNH_{31} is between 50 to 100. NNH: number needed to harm.

Specific Aim 3, to investigate patient factors that influence tooth brushing frequency benefit and risk in critically ill adults, is considered exploratory. Repeated measures linear mixed models will be fit treating dental plaque and mucosal inflammation as separate outcome variables (transformed if required), random group assignment as a 3-level ordinal predictor variable, and a range of possible patient-level predictors. Initially, bivariate correlations for potential continuous predictors and analysis of variance (ANOVA) for potential categorical predictors will be conducted to identify potential patient factors independently associated with dental plaque and mucosal inflammation. Interaction terms between tooth brushing frequency (scored 1, 2, or 3) and individual patient factors will be constructed to assess potential effect modification. The mixed models will be fit using the normal distribution, identity link, and specifying an autoregressive correlation structure. For assessment of safety, and assuming a sufficient number of HAIs for model conversion, generalized estimating equations will be fit in the same manner as the linear mixed models while specifying the binomial distribution, log link, and an autoregressive correlation structure. Through these analyses, we aim to gain insight as to specific patient factors (e.g. presenting oral health) that may modify the clinical effectiveness and safety profile associated with frequency of tooth brushing.

Statistical Power and Sample Size Justification. As described above, our primary aim is to evaluate whether a single daily tooth brushing regimen is clinically non-inferior (defined as an effect size of ≤0.50) to a regimen of brushing twice or 3 times daily. The test is considered one-sided as there is no known or postulated biological mechanism to expect a better clinical result (lower plaque score) with the once daily brushing regimen, while at the same time, no reason to expect a higher risk of serious adverse events with this regimen as compared to 3 times daily brushing. To be conservative, we assume that up to 40% of subjects enrolled with have missing data or not provide analyzable data due to intubation for less than 48 hours or less than 2 days follow-up measurement of dental plaque and mucosal inflammation after intubation; this is consistent with our previous and current projects. The non-inferiority trial is powered based on conventional guidelines of type I error rate (α) of 0.10 and 95% desired power, and specifying a 1-tailed tolerance limit for non-inferiority as an effect size of 0.50. With these conditions, a sample size of 69 subjects per group (i.e. testing group 1 versus 3) is required. This group size will also be sufficient if the non-inferiority test is not supported, and a secondary test of the 1 versus 2 times daily brushing regimens is necessary. For 3 groups, the required effective sample size is 207 (69 x 3 groups), which taking into account missing and non-usable outcome data results in a proposed total sample size of 345 subjects (i.e. 207 / 0.60). For specific aim #2, it is difficult to estimate the expected proportion of HAIs to occur in the respective treatment arms, particularly given the recent change in Centers for Disease Control and Prevention (CDC) National Healthcare Safety Network (NHSN) surveillance definitions for ventilator associated complications, including VAP.[55] If the proportion of subjects who experience HAI within one of the treatment groups (n=69) is 0.05, the corresponding 95% confidence interval would be 0.01 to 0.13, indicating reasonable precision.

Timeline

Table 3 Timeline for Proposed Study

Activity	Year / Months	Year 1 1-4	Year 1 5-12	Year 2 1-6	Year 2 7-12	Year 3 1-6	Year 3 7-12	Year 4 1-8	Year 4 9-12
Hire/ train personnel									
Database refinement									
Data entry									
Subject enrollment									
Data collection									
Data management									
Analysis of final results									
Final results manuscripts and final grant report									

XII. Data and Safety Monitoring

A Data and Safety Monitoring Plan (DSMP) will be implemented for the purpose of monitoring safety of the study, including but not limited to review of adverse events. Monthly DSMP meetings will include the PI, project director, biostatistician, data manager, and GRAs. The DSMP meetings will include review of all safety and adverse events data. Summary reports of data will be prepared by the data manager and project director. DSMP reports will consist of protocol compliance with inclusion/exclusion criteria, gender and minority and adverse events review. Written minutes of DSMB meetings with summaries of adverse events will be forwarded to the IRB and NIH as part of the annual reporting process. Research staff will report any adverse event immediately to the PI. Enrollment, study fidelity, and safety data will be reviewed regularly at study team monthly meetings. All adverse events will be reported to the TGH and USF IRBs, and the NIH, following institutional and NIH guidelines. We believe that the protocol is low risk and that this should be adequate.

XIII. Multi-center Studies

N/A

XIV. involvement of non-USF institutions/sites (domestic and foreign)

N/A

XV. Involvement of Independent Investigators

N/A

XVI. Human Subjects Instructions

A. Description

The proposed project will determine the optimal frequency of a tooth brushing intervention, by comparing three groups (once, twice, or three times daily), with the intent of informing the standard of nursing care for mechanically ventilated adults. As such, we believe this project meets the definition of "Human Subjects Research which involves an NIH-Defined Phase III Clinical Trial." Following obtainment of informed consent from the subject or LAR, subjects will be randomly assigned within 36 hours of intubation to one of three intervention groups which differ in frequency of tooth brushing (once, twice, or three times daily). The intervention, a standardized tooth brushing protocol developed and tested in the previous project, will be delivered by study personnel for 7 days, or until the subject is extubated if extubation occurs within the first 7 days. Data will be collected on study admission and every intervention day. For subjects who are extubated within the 7 day intervention period, data will also be collected on day 1,3, and 5 following extubation.

B. Subject Population

Subjects will be recruited at Tampa General Hospital (TGH). TGH is a private not-for-profit hospital and one of the most comprehensive medical facilities in West Central Florida serving a dozen counties with a population in excess of 4 million. We will enroll 345 critically ill, mechanically ventilated subjects, ages 18 and older. We anticipate that the subjects will be representative of the population, as described in Table 4.

Table 4. Characteristics of Study Population, Geographic Area and Hospital (2010 estimated data)

Characteristics	Hillsborough County, FL	Hospital (inpatient)
Gender		
Male	49.3%	43.9%
Female	50.7%	56.1%
Ethnic category		
Hispanic or Latino	23.4%	
Not Hispanic or Latino	76.6%	
Racial category		
American Indian/Alaska Native	0.4%	0.2%
Asian	3.0%	0.6%
Native Hawaiian/Pac Islander	0%	0%
Black / African American	16.0%	20.7%
White	71.2%	73.6%
More than 2/ Other / Unknown	9.3%	5.0%
Average age	37.1	48.8

Inclusion of Women, Minorities and Children

Inclusion of Women. TGH is a diverse clinical setting. Based on the patient characteristics of the study units in our current study, females make up approximately 40% of the population of each unit (about 10% less than the proportion of women in Hillsborough County, FL, or the entire TGH hospital population). We expect that recruitment of females will mirror these demographics. Our experiences during previous critical care studies show that recruitment of females is similar to that in the admission population. Therefore, it is anticipated that the subjects selected for this study will be representative in gender of the population of patients in the TGH ICUs. However, Dr. Kip and the Project Director will pay special attention to the admission of women to the unit. All women meeting inclusion criteria will be recruited. We will review the enrollment of women at our monthly study team meetings to ensure that we are meeting our target enrollment. Should we find that we are not meeting targets, we will immediately examine our enrollment data to determine if we have a recruitment issue or a temporary flux in unit admission of women. If it is a recruitment issue, we will analyze the enrollment data and discuss possible causes in order to determine a solution.

Inclusion of Minorities. TGH is also an ethnically and racially diverse clinical setting. Based on admission characteristics of the study units (Table 6: STICU and MRICU) minorities make up approximately 36% of the population of each unit. We expect that recruitment of minorities will also mirror these demographics. Our experiences during previous critical care studies show that recruitment of minorities is similar to that in the admission population. It is anticipated that the subjects selected for this study will be representative in ethnic background and race of the population of patients in the TGH ICUs. We intend to recruit minorities consistent with our accessible population. Dr. Kip and the Project Director will pay special attention to the admission of ethnic minorities to the units. All patients meeting inclusion criteria will be recruited. We will review the enrollment of racial and ethnic minorities at our monthly study team meetings to ensure that we are meeting our target enrollment. Should we find that we are not meeting targets, we will immediately examine our inclusion criteria in relation to the characteristics of subjects in the unit to determine if we have a recruitment issue or a temporary flux in minority admissions. If it is a recruitment issue, we will analyze the enrollment data and discuss possible causes in order to determine a solution.

Little evidence exists regarding clinically important sex/gender and/or race/ethnicity differences which could be associated with benefits or risks of varying tooth brushing frequency in mechanically ventilated adults. In addressing the exploratory study aim (to investigate patient factors that influence tooth brushing frequency benefit and risk in critically ill adults), we will include sex, ethnicity, and race as possible patient-level predictors in our analyses of risk and benefit.

Inclusion of Children**.** The target adult ICUs include patients who are 18 years or older. Children less than 18 years of age are not admitted to these ICUs and will not be recruited. Childhood dentition is significantly different from that of adults. The number of subjects ages 1-17 required for study enrollment to obtain adequate power to account for differences in dentition would be prohibitive and an adequate sample of children under 18 could not be obtained during the proposed study period.

C. RESEARCH MATERIAL

Data will be obtained from the patient's medical record, from assessment of the subject's mouth, and from biologic fluids (gingival crevicular fluid, GCF, collected on filter paper). On study admission, the following data will be collected: demographic data (age, gender, smoking status, reason for admission), severity of illness data (APACHE information), and a count of decayed, missing and filled teeth (DMFT). The following data will be collected every day through the first seven days of mechanical ventilation, or until extubated if extubation occurs within the first seven days: digital photos of all teeth (for use in UM-OHI dental plaque assessment), a sample of GCF collected on Periopaper (for GCF IL-1β assay), Systemic Inflammatory Response Syndrome criteria from the medical record, positive epidemiologic surveillance reports for enrolled subjects for VAE (including VAP), bacteremia, and sepsis from the TGH Epidemiology Department records. For subjects who are extubated during the first 7 days of mechanical ventilation, the same data collected during the study intervention period will also be collected after extubation on days 1,3,5 after extubation. Data regarding length of intubation, length of ICU stay and length of hospital stay will also be calculated from the medical record.

D. RECRUITMENT PLAN

All patients admitted to the adult Intensive Care Units at TGH who are intubated will be assessed for possible inclusion in the study. If a patient meets study criteria (age 18 or older, within 36 hours of initial intubation, have at least one tooth, and they or their legally authorized representative able to provide informed consent in English or Spanish) and has no exclusions (anticipation by the clinical provider of imminent patient death, or medical contraindication to tooth brushing), the potential subject will be approached for a discussion about willingness to participate. If the subject is unable to provide consent due to sedation or inability to communicate, the legally authorized representative (LAR) will be approached, the study will be reviewed and permission for the subject to participate will be obtained from the LAR; in these cases, we will attempt to obtain assent of the subject as well, and will make additional attempts to consent the subject directly as their condition improves. In our experience, potential subjects who are critically ill, especially during the first 36 hours of intubation, are always sedated to some degree, and their ability to communicate effectively is compromised by endotracheal intubation; for these reasons, permission for the subject's participation has generally been obtained from the LAR. However, there may be rare occurrences when potential subjects are adequately oriented and lucid to be able to provide informed consent. Therefore, we will use a 2 step procedure (developed by Fan et al[61]) to determine the patient's ability to provide informed consent. Step 1 is objective evaluation with the Richmond Agitation-Sedation Scale (RASS) and the Confusion Assessment Method for Intensive Care Unit (CAM-ICU). Patients who score -1, 0, +1 on the RASS indicating drowsy, alert, or restless respectively and are not delirious (CAM-ICU = No) will move to Step 2. Step 2 is assessment for competency using the MacArthur Competence Assessment Tool for Clinical Research: MacCAT-CR. This is the most widely used tool, and is the only tool that evaluates all four capacity domains (understanding, appreciation, reasoning, expression of a choice). Evaluation of Step 1 requires approximately 2-3 minutes, Step 2 requires approximately 15 minutes. Fan et al. found in consent evaluations of 150 ICU patients that 89% were sedated/delirious, and unable to provide consent; our experience in previous research is similar. Therefore, we expect that few potential subjects will move to Step 2. The LAR will then be contacted for those who fail Step 1 or Step 2. Subject's willingness to continue participation will be assessed on an ongoing basis throughout the project. We will follow the USF and TGH IRB Written Policies and Procedures, as well as the Florida regulations regarding appropriate use and definition of the LAR.

E. POTENTIAL RISKS

We anticipate minimal additional physical and psychological risks associated with this study. The intervention, tooth brushing, is routinely provided to critically ill patients by bedside nurses as part of clinical care. Organisms dislodged from dental plaque during tooth brushing might increase risk of HAIs if they are aspirated (contributing to VAP) or translocate into the bloodstream (contributing to bacteremia and sepsis); evaluation of safety is a specific aim of the proposed project. Data collection methods (including GCF collection by Periopaper and digital photographs of teeth) are non-invasive. Subjects may elect not to participate and may withdraw from the study at any time without affecting the care they receive.

F. RISK REDUCTION

The intervention will be interspersed with the care team's activities, and at no time will the study personnel delay or interfere with clinical care; if the needs and activities of providers are such that the study personnel are unable to access

the subject to provide interventions, the subject will be withdrawn from the study. At all times we will place the subject's need for medical treatment, and avoidance with interference with treatment, foremost.

In order to reduce risks to privacy of individuals or confidentiality of data, we will not include personally identifiable data in the database files. All computers and data files will be password protected, and data will be stored on the University server, with access only to study personnel, under the direct supervision of Dr. Kip. Because these subjects are receiving clinical care in the hospital, the attending clinical medical providers will be promptly informed of any adverse effects to the subjects; if an adverse event occurs, necessary interventions will be determined and delivered by the clinical providers.

G. Additional Safeguards If Any Participants Will Be Vulnerable

The trained Project Director or GRAs will review the potential subject's medical record, and if there are no exclusions, the subject will be invited to enroll. The subject and the subject's LAR will be provided with an oral explanation of the nature of the study and study information, in writing. The information will include all elements required for informed consent, and will include all pertinent contact information as well as information about withdrawal from the study. If at any time the subject or LAR does not wish to continue to participate in the study, no additional interactions with the subject will take place, no follow-up data will be collected and that subject's data forms will be destroyed.The study staff will work closely with the staff of the ICUs, the nurse managers and medical directors to ensure that all eligible patients have an equal opportunity for inclusion.

H. Confidentiality

Each participant will be assigned an arbitrary code number to ensure anonymity of the research data, and all research data will be maintained in locked file cabinets under the direct supervision of the PI. Study information obtained will be kept strictly confidential, and it will not be possible to identify any participant from the reports of publications that may result from this research.

I. Privacy

All interactions with subjects and LARs will be conducted in the subject's hospital room or a private area of the ICU. The information obtained will be kept strictly confidential, and it will not be possible to identify any participant from the reports of publications that may result from this research. Each participant will be assigned an arbitrary code number to ensure anonymity of the research data, and all research data will be maintained in locked file cabinets under the direct supervision of the PI.

J. Risk/Benefit

The risks are minimal, because tooth brushing is a commonly performed procedure, which is currently used in the clinical setting without any assessment of risk.

Benefits to participation include potential improvement in oral health, as all subjects receive the standardized tooth brushing protocol at scheduled intervals. Control of dental plaque and reduction of oral inflammation are likely to improve patient comfort, and may reduce systemic sequellae related to a nidus of inflammation in the mouth.

This study will result in a determination of the optimal frequency of tooth brushing in mechanically ventilated adults to maximize oral health benefits while minimizing systemic risks, thus optimizing patient outcomes as well as nursing effort. We anticipate that this knowledge will have immediate impact on bedside nursing practice, and that specific evidence-based guidance for oral care in mechanically ventilated adults will improve control of dental plaque and mucosal inflammation, with added benefit of improving patient comfort.

K. Compensation Plan

No subject compensation is planned.

L. Consent Issues

1. Consent Process

The Principal Investigator, Project Director or GRAs will recruit and enroll subjects. Study personnel will review each subject's medical record and confirm with the medical staff that the subject is age 18 or older and if there are no barriers to inclusion, the potential subject will be approached for enrollment. The subject's ability to participate in consent processes will be evaluated as previously described. The subject and the subject's legally authorized

representative (LAR) will be provided with an oral explanation of the nature of the study and study information in writing. In any instance where the potential subject's ability to comprehend and communicate his/her desires is in question, we will seek consent for continued participation from the LAR and will invite the research subject to provide his/her assent. Further, subject's willingness to continue participation will be assessed on an ongoing basis throughout the project. We will follow the USF and TGH IRB Policies and Procedures and Florida Code.

2. SPECIAL CONSENT PROVISIONS

We will obtain consent for ongoing study participation from the LAR for subjects unable to provide consent because of critical illness, emergent intubation and inability to communicate.

3. ASSENT PROCESS *for decisionally impaired subjects.*

We will evaluate the potential subject's level of orientation and response to our discussions of the study on a daily basis, and if the potential subject is able to respond in a manner that is clearly understandable, we will use the potential subject's own consent to continue participation. However, in any instance where the potential subject's ability to comprehend and communicate his/her desires is in question, we will seek consent for continued participation from the LAR and will invite the research subject to provide his/her assent. Further, subject's willingness to continue participation will be assessed on an ongoing basis throughout the project.

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