Understanding Treatment Effect Mechanisms of the CAMBRA Randomized Trial in Reducing Caries Increment

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Abstract

The Caries Management By Risk Assessment (CAMBRA) randomized controlled trial showed that an intervention featuring combined antibacterial and fluoride therapy significantly reduced bacterial load and suggested reduced caries increment in adults with 1 to 7 baseline cavitated teeth. While trial results speak to the overall effectiveness of an intervention, insight can be gained from understanding the mechanism by which an intervention acts on putative intermediate variables (mediators) to affect outcomes. This study conducted mediation analyses on 109 participants who completed the trial to understand whether the intervention reduced caries increment through its action on potential mediators (oral bacterial load, fluoride levels, and overall caries risk based on the composite of bacterial challenge and salivary fluoride) between the intervention and dental outcomes. The primary outcome was the increment from baseline in decayed, missing, and filled permanent surfaces (Δ DMFS) 24 mo after completing restorations for baseline cavitated lesions. Analyses adjusted for baseline overall risk, bacterial challenge, and fluoride values under a potential outcome framework using generalized linear models. Overall, the CAMBRA intervention was suggestive in reducing the 24-mo DMFS increment (reduction in Δ DMFS: -0.96 ; 95% confidence interval [CI]: -2.01 to 0.08 ; $P = 0.07$); the intervention significantly reduced the 12-mo overall risk (reduction in overall risk: -19% ; 95% CI, -7 to -41% ; $P = 0.005$). Individual mediators, salivary $\log_{10}$ mutans streptococci, $\log_{10}$ lactobacilli, and fluoride level, did not represent statistically significant pathways alone through which the intervention effect was transmitted. However, 36% of the intervention effect on 24-mo DMFS increment was through a mediation effect on 12-mo overall risk ($P = 0.03$). These findings suggest a greater intervention effect carried through the combined action on multiple aspects of the caries process rather than through any single factor. In addition, a substantial portion of the total effect of the CAMBRA intervention may have operated through unanticipated or unmeasured pathways not included among the potential mediators studied.

Keywords: caries management, caries risk, lactobacillus, mediation analysis, *Streptococcus mutans*, salivary fluoride

Introduction

In many dental studies, an intervention is designed to change postrandomization (intermediate) variables (e.g., knowledge, behavior, or biological factors) under the hypothesis that changing those intermediate variables will lead to improvement in final dental outcomes (MacKinnon and Luecen 2011). In such studies, researchers are interested in not only whether the intervention works to improve final outcomes but also how it works (i.e., mechanisms). That is, interest lies in whether, and how much, the intervention affects the outcome through either (1) measured intermediate variables, as designed, or (2) direct intervention effects on the outcome involving unmeasured or unknown intermediate variables. Analysis of mediation pathways obligates researchers to be explicit about the

theoretical basis that motivates a particular intervention and creates opportunities to test competing theories about mechanisms of action (MacKinnon and Luecen 2011). Enhanced understanding of intervention mechanisms and the particular mediating variables involved would allow greater opportunities to tailor evidence-based practices to specifically targeted populations of interest.

The standard analysis, comparing the dental outcomes between treatment and control groups, provides the overall treatment effect estimate but does not provide information

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Table 1. Participant Inclusion and Exclusion Criteria in the CAMBRA Trial.

Inclusion criteria	Ability to speak and understand English; 18 y of age or older; planning to stay in the immediate area for the next 3 y; a minimum of 16 teeth; willing to have needed dental radiographs every 2 y; at least 1 and up to 7 cavitated carious teeth; no moderate or severe periodontal disease needing periodontal surgery or chemotherapeutic agents; voluntarily provided written informed consent
Exclusion criteria	Significant past or current medical history of conditions that may affect oral health or oral flora (i.e., diabetes, HIV, heart conditions that require antibiotic prophylaxis); use of medications that may affect the oral flora or salivary flow (e.g., antibiotic use in the past 3 mo, drugs associated with dry mouth/xerostomia); complex dental need; frequent periodontal maintenance; another household member participating in the study; drug or alcohol addiction, or other conditions that may decrease the likelihood of adhering to the study protocol

on the mechanism or pathway associated with the treatment effect. Even if a treatment does not have a significant effect, a positive mediation effect through intermediate variables could possibly cancel a direct negative effect through other pathways, leading to no overall effect.

The Caries Management by Risk Assessment (CAMBRA) randomized controlled clinical trial (R01 DE 012455; Featherstone et al. 2012) was conducted at the School of Dentistry, University of California, San Francisco, with institutional review board approval. Based on the hypothesis that caries arises when pathological factors (e.g., oral bacteria, such as mutans streptococci [MS] and lactobacilli [LB]) outweigh protective factors (e.g., salivary fluoride, saliva flow; ten Cate and Featherstone 1991; Featherstone 2003), the study aimed to assess whether combined antibacterial and fluoride therapy based on risk assessment has beneficial effects on preventing new caries over 24-mo follow-up in adults with 1 to 7 baseline cavitated teeth. The intervention sought to reduce the pathological factors and to increase protective factors in high-risk participants with a high bacteria level and low salivary fluoride. The primary analyses showed that the intervention group had a statistically significantly lower caries risk at follow-up and suggested a lower average caries increment (Δ DMFS) compared with control over 2 y (Featherstone et al. 2012). Of interest is whether this overall intervention effect was due mainly to lowered overall caries risk, bacteria reduction, or fluoride increase, as anticipated. If observed, mediation through these mechanisms would provide further evidence for focusing future caries prevention efforts on these components. Therefore, in this article, we will examine if, and how much, the intervention reduced caries increment through actually lowering caries risk, reducing bacterial levels, or increasing salivary fluoride, as opposed to through other unknown factors/mechanisms.

Subjects and Methods

Subjects

The CAMBRA trial enrolled 231 adults with 1 to 7 baseline cavitated carious teeth at the School of Dentistry Student

Dental Clinics from 1999 to 2001 (see Table 1 for inclusion and exclusion criteria); 115 were randomized to control and 116 to the intervention group. Included for analysis in this article were those 109 participants (47% of enrolled participants; 52 in the control group and 57 in the intervention group) who completed the CAMBRA trial, with final clinical examinations completed from 2001 to 2004. Table 2 shows the baseline characteristics for all participants and participants who completed the trial.

Treatment Groups

Both the intervention and control groups received initial caries removal and restorations following the baseline examination; salivary assays for assessment of MS, LB, and fluoride at baseline, when initial restorations were complete, and every 6 mo thereafter; and radiographs and dental examinations at the beginning and end of the study. In addition, after initial caries removal and restoration, participants in the control group received conventional treatment per usual clinical practices (e.g., oral hygiene instruction, periodic dental cleaning and oral examination scheduled every 6 mo, radiographs scheduled every 24 mo, and restorative treatment as needed), while participants in the intervention group received a targeted, combined antibacterial (0.12% chlorhexidine gluconate mouth rinse) and fluoride therapy (1,100 ppm sodium fluoride toothpaste, 0.05% sodium fluoride mouth rinse, and topical 1.1% NaF gel application) based on their individual risk assessments. Demographics, clinical variables, and outcomes were measured at baseline and at follow-up intervals (Tables 2 and 3).

Mediators of Interest

Based on the theoretical mechanism of the CAMBRA intervention to increase protective factors and decrease bacterial challenge (Figure), we are interested in overall risk, bacterial challenge (salivary MB and LB levels), and salivary fluoride value as possible mediators. The overall risk was predefined as low or high based on a composite measure of bacteria challenge and salivary fluoride levels (Table 4) based on

Table 2. Baseline Characteristics of All Participants and Participants Who Completed the Trial (Featherstone et al. 2012).

	All Participants		Participants Who Completed the Trial ^a	
	Control (n = 116)	Intervention (n = 115)	Control (n = 52)	Intervention (n = 57)
Age, y, mean $\pm$ standard deviation	38.4 $\pm$ 13.5	36.9 $\pm$ 13.2	40.9 $\pm$ 14.8	39.2 $\pm$ 14.7
Sex: female, n (%)	69 (59)	69 (60)	32 (62)	38 (67)
Race/ethnicity, n (%)				
Asian, non-Hispanic	8 (7)	15 (13)	8 (15)	14 (25)
Black, non-Hispanic	6 (5)	8 (7)	6 (12)	8 (14)
White, non-Hispanic	18 (16)	28 (24)	18 (35)	27 (47)
Hispanic	13 (11)	7 (6)	13 (25)	6 (11)
Other/multiracial, non-Hispanic	4 (3)	2 (2)	3 (6)	1 (2)
Refused/missing	67 (58)	55 (48)	4 (8)	1 (2)
Education, n (%)				
High school	26 (22)	30 (26)	10 (19)	10 (17)
College	65 (56)	58 (50)	26 (50)	30 (53)
Graduate/professional	25 (22)	27 (23)	16 (31)	17 (30)
Works in San Francisco, n (%)	80 (69)	85 (74)	42 (81)	42 (74)
Last dental visit, n (%)				
<6 mo prior	27 (24)	32 (28)	16 (32)	16 (29)
6 to 11 mo prior combine <12 mo	10 (9)	23 (20)	4 (8)	90 (18)
12 to 23 mo prior	26 (23)	20 (18)	10 (20)	9 (16)
24 to 35 mo prior combine 24+ mo	22 (19)	14 (12)	11 (22)	7 (13)
36+ mo prior	29 (25)	25 (22)	9 (18)	13 (23)
Ever had professional cleaning, n (%)	98 (90)	100 (91)	46 (94)	48 (89)
Brushed 2+ times yesterday, n (%)	89 (77)	85 (74)	40 (77)	42 (74)
Used fluoride toothpaste, n (%)	87 (76)	79 (69)	39 (75)	40 (70)
Fair/poor oral health, n (%)	61 (53)	62 (54)	22 (42)	22 (39)
Drank alcohol in past week, n (%)	61 (53)	57 (50)	25 (50)	27 (47)
Smoked cigarette $\leq$ 30 d, n (%)	34 (29)	31 (27)	6 (11)	8 (14)

^aCompared with those who dropped out, participants with complete data were more likely at baseline to work in San Francisco, be more educated, have better self-rated oral health status, and report less alcohol and tobacco use (all $P < 0.05$; Featherstone et al. 2012). However, baseline characteristics between the intervention and control groups for participants with complete data were not significantly different ($P = 0.305$), indicating that the 2 comparison groups were balanced in baseline covariates and that ignorability of treatment is a reasonable assumption.

previous findings (Krasse 1988; Leverett, Featherstone, et al. 1993; Leverett, Proskin, et al. 1993).

Outcome of Interest

The primary outcome of interest in this study was the increment from baseline in the number of decayed, missing, and filled permanent surfaces (Δ DMFS) 24 mo after initial restorations were completed for baseline lesions. DMFS increment is a nonnegative integer count or a zero-inflated count if many participants have no new DMFS, with higher values indicating worse dental outcomes.

Effects of Interest

Overall Effect. The overall effect of an intervention is the total effect of the intervention on the outcome compared with

control, no matter whether the effect operates through the mediator of interest. The overall effect of the intervention is the sum of 2 components: (1) the effect of the intervention through the mediator of interest, called the *indirect or mediation effect*, and (2) the effect of the intervention around the mediator of interest, called the *direct effect* of the intervention (Figure). The direct effect may operate through unknown or unmeasured variables but not through the mediator of interest. Standard analysis estimates the average overall effect of an intervention compared with control without effect decomposition into the component indirect and direct effects.

Indirect or mediation effect. The indirect or mediation effect of an intervention is the influence of the intervention through the mediator of interest. This effect can be conceptualized as the change in the outcome due exclusively to the effect of the intervention on the mediator. In other words, it

Table 3. Bacterial Challenge, Salivary Fluoride Concentration, High Overall Caries Risk, and DMFS Increment by Treatment Group.

	Mediator			
	High Overall Caries Risk, %	Log ₁₀ MS, mean ± SD	Log ₁₀ LB, mean ± SD	Fluoride ppm, mean ± SD
Baseline				
Intervention	93	4.27 ± 1.42	3.60 ± 1.95	0.03 ± 0.02
Control	90	4.51 ± 1.29	3.71 ± 1.98	0.03 ± 0.02
P	0.74	0.37	0.76	0.73
12 mo				
Intervention	67	3.26 ± 1.95	2.92 ± 2.10	0.08 ± 0.14
Control	88	4.58 ± 1.49	3.28 ± 2.00	0.04 ± 0.03
P	0.01	<0.01	0.25	0.02
Mediation effect				
Mean (95% CI)	−0.38 (−0.82 to −0.03)	−0.31 (−0.90 to 0.11)	0.00 (−0.17 to 0.18)	0.02 (−0.25 to 0.30)
P	0.03	0.17	0.99	0.88
DMFS Increment = 0, n (%)		DMFS Increment, mean ± SD		
Outcome at 24 mo				
Intervention	7 (12.3)		3.5 ± 3.5	
Control	4 (7.7)		4.6 ± 4.1	
P	0.32		0.02	

CI, confidence interval; DMFS, decayed missing filled tooth surface index; LB, lactobacilli; MS, mutans streptococci, SD, standard deviation.

Table 4. Low and High Overall Caries Risk Predefined as a Function of log₁₀ MS, log₁₀ LB, and Salivary Fluoride (Featherstone et al. 2012).

log ₁₀ LB (CFU/mL)	log ₁₀ MS (CFU/mL)		
	<4	4 to 5.9	≥6
Low salivary fluoride: [F] < 0.03 ppm			
<1	Low risk	High risk	High risk
1 to 2.9	High risk	High risk	High risk
≥3	High risk	High risk	High risk
Medium salivary fluoride: 0.03 ppm ≤ [F] < 0.079 ppm			
<1	Low risk	Low risk	High risk
1 to 2.9	Low risk	High risk	High risk
≥3	High risk	High risk	High risk
High salivary fluoride: [F] ≥ 0.08 ppm			
<1	Low risk	Low risk	High risk
1 to 2.9	Low risk	Low risk	High risk
≥3	Low risk	High risk	High risk

LB, lactobacilli; MS, mutans streptococci.

is the expected difference in the outcome if the intervention changed the mediator variable but with no other alteration in treatment status or in alternative variables through which the intervention might act. This effect provides information as to how much, if any, of the overall intervention effect on the outcome can be attributed to the intervention influencing the mediator, as opposed to the intervention effect through other pathways.

Direct effect. The direct effect of an intervention is the influence of the intervention around (not through) the mediator of interest. This effect can be conceptualized as the change in the outcome due to any and all actions of the intervention that do not involve altering the mediator of interest. This effect is the difference in outcome expected between intervention and control if the mediator had been fixed so that no treatment effect could be attributed to it.

Figure. Theoretical mechanism, total effect, direct effect, and mediation effect of the CAMBRA intervention. CAMBRA, Caries Management By Risk Assessment; CI, confidence interval; DMFS, decayed missing filled tooth surface index; LB, lactobacilli; MS, mutans streptococci.

A significant direct effect of an intervention indicates that the intervention operates to affect the outcome through 1 or more alternative pathways that do not include effects on the mediator of interest.

Power Estimation

The CAMBRA trial was designed to have a power of 90% with a final evaluable sample size of 122 to detect an overall difference in caries incidence proportions of 0.60 and 0.30 (Featherstone et al. 2012). Given 109 participants who completed the study, there would be 66% power to detect an overall effect of 1.7 DMFS increment, with 40% power for a direct effect of 1.1 DMFS increment and 57% power for a mediation effect of 0.6 DMFS increment.

Statistical Methods

Previously, linear structural equation models (LSEM) have been used to examine the direct and indirect effects of an intervention or of measured social status on oral health (Donaldson et al. 2008; Tu et al. 2008). Instead of clearly defining causal mediation effects independent of statistical models, LSEMs interpret coefficient estimates only within a particular statistical model as causal mediation effects. Although LSEMs are usually presented along with a set of strong assumptions on all the variables in the system, the assumptions necessary to identify direct and indirect effects to which causal interpretations can be ascribed are rarely articulated explicitly. The LSEMs are also difficult to extend to nonlinear models. (See VanderWeele 2012 for a

more detailed discussion on structural equation models.) Various methods based on the potential outcome framework (Rubin 2004; Albert 2008; Imai et al. 2010; Vansteelandt 2010) have been recently proposed for mediation analysis. Compared with conventional mediation analysis (such as LSEM), popularized since Baron and Kenny (1986), these newer methods first clearly define causal mediation effects independent of particular statistical models and then specify assumptions to identify the effects. These methods can also be extended to nonlinear models for general outcomes (other than normally distributed outcomes) when linear models do not fit the data well. Dental outcomes such as caries incidence and DMFS increment are often binary and count or zero-inflated count variables with a relatively large portion of participants with values of zero to indicate no new DMFS; such data are not normally distributed, so linear models usually do not fit well. Albert and Nelson (2011), Albert (2012), and Wang et al. (2013) have applied the potential outcome-based methods to binary and count dental outcomes. In this article, we used a method for mediation analysis in which Cheng et al. (unpublished data, 2014) extended the approach of Imai et al. (2010) specifically to assess count and zero-inflated count dental outcomes.

The method (Imai et al. 2010; Cheng et al. unpublished data, 2014) assumes sequential ignorability of intervention and mediator given baseline covariates; that is, the intervention and mediator are unrelated to confounders after adjusting for baseline covariates. Since the intervention was randomized in the CAMBRA trial, assuming ignorability of the intervention is straightforward in this study. Although the postbaseline mediators (overall risk, bacteria load, and

salivary fluoride at 12 mo) were not randomized, controlling for relevant baseline covariates allows the assumption of no unmeasured confounding on mediators to be reasonable.

The analyses were based on the quasi-Bayesian Monte Carlo approximation (King et al. 2000) and involve 5 steps (Imai et al. 2010; Cheng et al. unpublished data, 2014). Briefly,

1. Fit the mediator and outcome models with observed data.

For the continuous mediators (bacterial challenge and salivary fluoride concentration), a linear model was fitted for the mediator. For the binary mediator overall caries risk (high or low), a logistic regression model was fitted for the mediator. All of the mediator models included the treatment assignment as the independent variable and baseline value of the mediator as a covariate. For the outcome DMFS increment, generalized linear models for Poisson, negative binomial (NB), zero-inflated Poisson (ZIP), and zero-inflated negative binomial (ZINB) were fitted, each with the treatment assignment and mediator as independent variables and baseline value of the mediator as a covariate. The Vuong test (Vuong 1989) was used to compare the 4 outcome models (Poisson, NB, ZIP, and ZINB) to select the best model for the final analysis.

2. Simulate model parameters (coefficients) from their sampling distributions.
3. Given the coefficients randomly drawn from their sampling distributions in (2), (a) simulate potential values of the mediator based on the mediator model, (b) simulate potential outcomes based on the outcome model and simulated potential values of the mediator from (a), and (c) compute the direct, mediation, and overall effects of the intervention.
4. Perform Monte Carlo replications by repeating steps (2) and (3) 1,000 times each.
5. Compute the point estimates and 95% confidence intervals for direct, mediation, and total effects of the intervention and corresponding *P* values based on the results from the 1,000 repetitions.

Analysis was completed using R software, version 3.0.3 (www.r-project.org).

Results

Among the 115 participants randomized to control and 116 to intervention, 52 (45%) participants in the control group and 57 (49%) in the intervention group completed the CAMBRA trial and final dental examination through the 24-mo follow-up period and were included in this analysis. Table 2 shows the major baseline characteristics of all

participants and participants who completed the trial. Compared with those who dropped out, participants with complete data were more likely at baseline to work in San Francisco (77.1% vs 66.4%), be more educated (college and above: 81.7% vs 70.5%), have better self-rated oral health status (good and above: 59.6% vs 35.3%), and report less alcohol (never: 19.6% vs 9.1%) and tobacco use (never: 87.2% vs 58.2%; all *P* < 0.05; Featherstone et al. 2012). However, baseline characteristics between the intervention and control groups for participants with complete data were not significantly different (*P* = 0.305), indicating that the 2 comparison groups were balanced in baseline covariates and that ignorability of treatment is a reasonable assumption.

Table 3 shows the intervention effect on potential mediators at 12 mo. The mean $\log_{10}$ MS at 12 mo was reduced about 1.0 CFU/mL from baseline in the intervention group but remained about 4.5 CFU/mL in the control group. The mean $\log_{10}$ LB at 12 mo was reduced about 0.7 CFU/mL from baseline in the intervention group and about 0.4 CFU/mL in the control group. The mean salivary fluoride concentration was increased about 0.05 ppm in the intervention group and about 0.01 ppm in the control group. The intervention group had a significantly lower MS level (*P* = 0.0002) and higher salivary fluoride level (*P* = 0.0233) than the control group at 12 mo, but there was no statistically significant difference in LB level between the 2 groups. The percentage of participants classified in the high-caries-risk group decreased from most participants at baseline (93%) to two-thirds (67%) at 12-mo follow-up in the intervention group but remained high (12-mo follow-up: 88%) in the control group (Table 3). The intervention group had a significantly lower percentage of participants classified as high overall risk at 12 mo than the control group (*P* = 0.0046).

At the end of the trial, at 24 mo, 12% of participants in the intervention group did not have any increment in DMFS, compared with about 8% in the control group (Table 3). The intervention group had mean DMFS increment about 1 surface fewer (3.5) than the control group (4.6), but the mean change in DMFS at 24 mo was not significantly different between the 2 groups in the primary analysis based on the extended Mantel-Haenszel test (*P* = 0.101; Featherstone et al. 2012). The DMFS increment in the 2 groups was not normally distributed (Kolmogorov-Smirnov test, *P* < 0.01), indicating a linear model assuming normality may not be a good fit for the data and that count data methods may fit better.

Mediation analyses were conducted following the 5 steps discussed in the Statistical Methods section with the mediators of interest. Generalized linear models were fitted separately for the DMFS increment using Poisson, NB, ZIP, and ZINB distributions. The NB model fit best by the Vuong test for all the mediators (e.g., goodness of fit for overall caries risk: NB > Poisson, *P* = 0.004; NB > ZIP, *P* = 0.006; NB > ZINB, *P* = 0.310). Table 3 shows the mediation effects

through overall caries risk, $\log_{10}$ MS, $\log_{10}$ LB, and salivary fluoride concentration at 12 mo, in which only the mediation effect through 12-mo overall caries risk was statistically significant ($P = 0.03$).

The Figure shows the pathways for direct, mediation, and total effects of the intervention through 12-mo overall caries risk. The total effect of the CAMBRA intervention was -0.96 tooth surfaces, with a 95% confidence interval (CI; -2.01 to 0.08), indicating that the intervention was suggestive at reducing DMFS increment by almost 1 surface on average compared with control ($P = 0.07$) in adults who had 1 to 7 baseline cavitated teeth and received the initial caries removal and restorations. The direct effect of the CAMBRA intervention that excluded any effect on 12-mo overall caries risk was -0.58 tooth surfaces (95% CI, -1.57 to 0.42) but was not statistically significant ($P = 0.25$). The mediation (indirect) effect of the CAMBRA intervention through reduction in 12-mo overall caries risk was statistically significant with -0.38 tooth surfaces ($P = 0.03$; 95% CI, -0.82 to -0.03), meaning that approximately 36% (median of $\left(\frac{\text{mediation effect}}{\text{total effect}}\right)\%$ over 1,000 Monte Carlo replications) of the intervention effect on reducing 24-mo DMFS increment was through its mediation effect on the 12-mo overall caries risk (Figure). No other statistically significant mediation effect was found in this study (Table 3).

Discussion

Mediation models have attracted considerable interest in health research in general and more recently in oral health research in particular (Garcia 2011), to better understand the mechanisms of how an intervention works to improve outcomes (Donaldson et al. 2008; Tu et al. 2008). However, the conventional mediation analysis largely based on linear models may not fit many dental outcomes, which are frequently count or zero-inflated count data. This article illustrates a useful new extension of a method based on the potential outcome framework (Albert 2008; Imai et al. 2010) for dental data (Cheng et al. unpublished data, 2014) to further understand the mechanism of the CAMBRA intervention.

The CAMBRA intervention was based on the hypothesis that a combined antibacterial and fluoride treatment would reduce oral bacterial challenge and increase salivary fluoride level (i.e., reduce overall risk), which would then lead to reduced DMFS increment. Perhaps because of a limited sample size, the mediation analyses conducted in this study failed to show a statistically significant mediation effect through bacterial challenge or salivary fluoride level individually. In contrast, significant mediation was found through the overall risk at 12 mo, accounting for 36% of the total effect. The overall risk itself is an a priori composite measure combining

the effects through multiple mediators ($\log_{10}$ MS, $\log_{10}$ LB, and salivary fluoride), suggesting that the CAMBRA intervention transmitted more of its anticaries effect through combined action on multiple mediators than through any single variable. In the future, a larger study would be needed to understand other potential pathways not included in this study that could account for the remaining total effect of the CAMBRA intervention on 24-mo DMFS increment. While we can only speculate as to the nature of these pathways (e.g., ecological shifts in biofilm-residing plaque bacteria or behavioral changes triggered by following the intervention protocol), an important implication of these findings is that much of the total effect of the CAMBRA intervention was potentially transmitted through mediators that were unmeasured or unanticipated, speaking to the complexity and multifactorial nature of the caries process. Future mediation analyses applied to other caries prevention trials could help elucidate such mechanisms, provided that investigators have collected detailed data on potential mediators.

Similar to the conventional mediation analysis methods, the method used in this study assumes sequential ignorability of the intervention and mediator; that is, the intervention and mediator are not associated with confounders. Although the ignorability of the intervention is often satisfied in a trial by random assignment, we must plausibly assume that ignorability of the mediator is achieved after controlling for baseline covariates. However, it is possible that unmeasured confounding could have some influence on our estimates. In addition, future directions include extending the methodology to accommodate multiple mediators and applying those new methods to these data.

In conclusion, our study suggests that the CAMBRA intervention transmitted more of its anticaries effect through the combined action on multiple mediators than through any single variable. This finding is consistent with studies on multifactorial health problems, which suggest that a program that addresses a variety of risk and protective factors in multiple domains would have a greater impact on improving outcomes than actions targeting any single factor (Office of the Surgeon General et al. 2001; NSW Department of Community Services 2007; Saminsky 2010). Likewise, future anticaries interventions and clinical approaches are likely to be most efficacious when designed to operate through diverse mediating pathways, consistent with the complexity of the caries process.

Author Contributions

J. Cheng, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; B.W. Chaffee, contributed to data acquisition and interpretation, critically revised the manuscript; N.F. Cheng, contributed to data analysis and interpretation, critically revised the manuscript; S.A. Gansky, J.D.B. Featherstone, contributed to

conception, design, data acquisition, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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