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Vibratory Stimulus Reduces *in vitro* Biofilm Formation On Tracheoesophageal Voice Prostheses§

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ABSTRACT

Biofilm has been amply demonstrated as the primary cause for tracheoesophageal prosthesis (TEP) failure in patients using them for alaryngeal speech.[2] Vibration reduces biofilm in the oral cavity in many oral health studies.[11-12] We hypothesized that biofilm formation will be reduced on TEPs when vibration is applied compared to controls in a dynamic *in vitro* model simulating the tracheoesophageal puncture site.

16 *ex vivo* TEPs were cleansed and sterilized prior to random placement by length in two parallel modified Robbins devices. Each device was seeded with polymicrobial oral flora on day 1 and received basal artificial salivary flow continuously with three growth medium meals daily. One device was randomly selected for 2 minutes of vibration applied to each TEP before and after meals for 5 days. The TEPs were explanted and the biofilm cultured. This process was repeated after study arm crossover.

Vibrated TEPs had a significant biofilm reduction of 5.56-fold compared to controls ($p < 0.001$). Significant reductions were observed within length subgroups. Application of vibration around meals significantly reduces biofilm accumulation on TEPs *in vitro*. Further studies will need to determine if this correlates with longer device lifespan *in vivo*.

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INTRODUCTION

Alaryngeal speech has challenged patients and surgeons from the time of the first laryngectomy; nearly a century later, the first tracheoesophageal voice prosthesis (TEP) was developed.[1] Significant frustration exists with TEP failure due to the effects of biofilm accumulation.[2] Biofilm represents the adherence and growth of *Candida*, *Staphylococcus*, *Streptococcus*, & *Rothia* species, among others, on organic and inorganic surfaces like implantable biomedical devices.[3-7] Mean TEP lifetime averages 3 to 5 months for most patients before biofilm-related malfunction, but some fail as often as every 3 to 4 weeks.[8]

Attempts to decrease biofilm have included enzymes, biosurfactants, and chemical modifications with varying degrees of success.[3-7,9-10] An as yet unexplored way to reduce TEP biofilm is direct mechanical vibration. Multiple reports detail the use of mechanical and acoustical vibration to diminish biofilm in the oral cavity.[11-12] The ubiquity and benignity of electric sonicating toothbrushes offers further evidence of cultural familiarity with using vibration to prevent biofilm associated dental caries.[12] Vibration offers the possibility of reducing microbial adherence and disrupting their elaboration of biofilm.

The hypothesis of this current research posits that mechanical vibration applied directly to the TEP will reduce the accumulation of biofilm on TEPs in colony forming units (CFU) per milliliter (mL) in a dynamic microcosmic system utilizing a proprietary modified Robbins device (MRD).

METHODS AND MATERIALS

16 *ex vivo* Atos Medical® Provox®2 22.5 French indwelling silicone voice prostheses with lengths of 6 millimeter (mm), 8mm, and 10mm were individually scrubbed, sonicated, disinfected for 30 minutes (min) in 70% ethanol, and dried in aseptic conditions prior to random placement by length into MRDs particularly emulating the *in vivo* TEP site (Figure 1).

The experimental setup is demonstrated in Figure 2. The entire system of flasks, tubing, and MRDs was autoclaved to ensure internal sterility prior to starting the experimental run. The MRDs received basal diurnal and nocturnal salivary flow and 3 simulated growth medium (GM) "meals" daily. On the 1st day, the system was inoculated with oral flora during the 3 meals. Over the subsequent 4 days, sterile GM meals were provided.

Vibration was applied to all TEPs in one randomly selected MRD at 260hz for 2 min duration before and after each meal. After 5 days, the TEPs were aseptically removed and individually vortexed and sonicated to release the biofilm into individual suspensions before serial dilution and duplicate culturing onto 2 blood agar plates with a spiral plating apparatus. CFU/mL for each plate were determined with an automated colony counter utilizing the average of 3 measurements each for the 2 blood agar plates of the 1:1,000 dilution. The entire experimental system was then disassembled, decontaminated with 70% ethanol, scrubbed, rinsed with deionized water and reassembled. The TEPs underwent an exact duplicate run and culturing with crossover of which MRD's TEPs received vibration.

*For lack of blood agar plates, size 10mm vibratory TEPs were unable to be cultured/counted.

Figure 1: Proprietary Modified Robbins Device

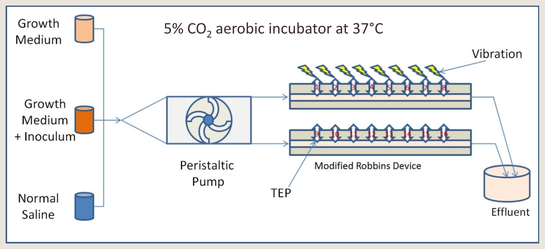

Figure 2: Experimental Setup
Growth Medium = 1:1 Yeast Extract Peptone-Dextrose: Brain-Heart Infusion
Inoculum = *C. albicans & tropicalis, S. salivarius, R. dentocariosa, S. aureus & epidermidis*.

RESULTS

- Comparing all vibratory to all non-vibratory TEPs for both runs collectively, a significant 5.56-fold reduction in mean CFU/mL was observed (Table 1, Figure 3).
- 1st run showed a significant 3.6-fold reduction between the mean CFU/mL ($2.0 \times 10^6 \pm 0.21$ CFU/mL (non-vibratory group) versus $0.55 \times 10^6 \pm 0.10$ CFU/mL (vibratory group); $t(13) = -6.39$, $p = 0.000024$).
- 2nd run showed a significant 9.4-fold reduction between the non-vibratory and vibratory mean CFU/mL with analysis following a natural log transformation: 14.4 ± 0.28 ln(CFU/mL) versus 12.2 ± 0.25 ln(CFU/mL); ($t(14) = 5.70$, $p = 0.000055$).
- For all length subgroups, significant mean CFU/mL reductions were observed; with a trend toward higher reduction in larger TEP lengths (Table 1, Figure 4).
- One-way ANOVA comparing mean CFU/mL showed no significant difference between length subgroups in vibratory ($F2,13 = 1.55$, $p = 0.25$) & non-vibratory groups ($F2,12 = 3.84$, $p = 0.052$).

Table 1

Comparison of mean CFU/mL formed in vibratory and non-vibratory groups based on TEP length.

TEP length (mm)	Vibratory group (CFU/mL)	Non-vibratory group (CFU/mL)	(n)	t-value	Sig.
6	$0.30 \times 10^6 \pm 0.11 \times 10^6$	$1.5 \times 10^6 \pm 0.11 \times 10^6$	16	t(7) = -5.64	0.00078⋆
8	$0.44 \times 10^6 \pm 0.33 \times 10^6$	$2.5 \times 10^6 \pm 0.33 \times 10^6$	12	t(5) = -3.10	0.027⋆
10	0.79×10^6 (n=1)	5.7×10^6 (n=1)	2	‡	‡
Combined	$0.39 \times 10^6 \pm 0.18 \times 10^6$	$2.2 \times 10^6 \pm 0.18 \times 10^6$	30	t(14) = -4.86	0.00025⋆

Note. The *combined* group represents cumulative data including all TEP lengths
Note. TEP = tracheoesophageal voice prosthesis; CFU = colony forming unit
Note. P-values (Sig.) are derived from paired samples t-tests comparing vibratory and non-vibratory groups within each row. SEM of the differences between paired samples is used to express variability

‡ Statistical analysis could not be performed due to insufficient sample size in 10mm TEP group
⋆ Denotes statistical significance at 95% confidence interval ($p < 0.05$)

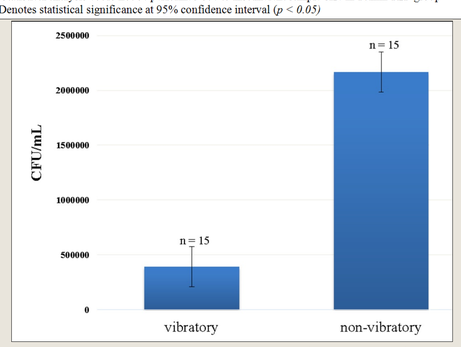

Figure 3: Paired vibratory & non-vibratory groups with all TEP lengths & runs included (excluding unpaired 1st run vibratory 10mm TEP). Vibratory mean CFU/mL ($0.39 \times 10^6 \pm 0.072$) is significantly different than the non-vibratory ($2.2 \times 10^6 \pm 0.41$); t (14), $p = 0.00025$. Error bars represent SEM of the differences between paired samples.

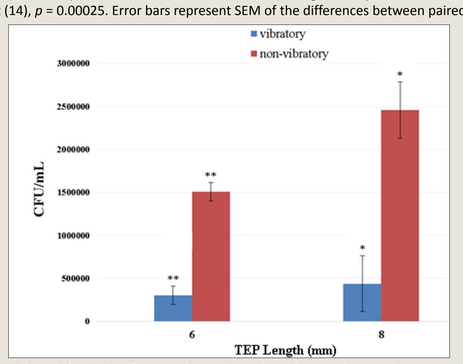

Figure 4. TEP length & CFU/mL for paired samples within vibratory & non-vibratory groups. Significant differences exist between vibratory & non-vibratory groups with 6mm & 8mm TEPs. * = $p < 0.05$; ** = $p < 0.001$ comparing vibratory & non-vibratory groups at each TEP length. Error bars represent SEM of the differences between paired samples.

DISCUSSION

As TEP length increased, the proportional significant reduction related to vibration also increased; a 5.0 fold reduction for 6mm TEPs & a 5.6 fold reduction for 8mm TEPs. This may relate to stasis of GM and bacteria within the longer TEP shaft by capillary forces. Prolonged stasis of vibrated biofilm may allow more rapid re-accumulation in larger TEPs. Analysis revealed that within both the vibratory and non-vibratory groups, length did not significantly affect CFU/mL cultured. Therefore, mechanical vibration appears to have the strongest effect among the potential variables influencing biofilm formation in this study.

Study strengths include parallel arrangement of MRDs within a run and the crossover of treatment arms between runs ; confirming that biofilm reduction was not likely related to local growing condition variability nor intrinsic susceptibility for biofilm formation by TEP subsets. A potential study limitation was the relative brevity of each experimental run compared to the minimum expected TEP device lifespan of 4-8 weeks. Many biofilm studies, however, run over 3-8 days with biofilm CFU/mL reduction on the order of the 10^6 observed in our study, thus we posit our experimental duration was appropriate.[4,12]

CONCLUSIONS

Vibration similar to what has been used in oral health industry to prevent dental caries for years has been shown in this study to significantly reduce biofilm formation on TEPs *in vitro*.

Research in the clinical setting will be necessary to demonstrate that this *in vitro* reduction in biofilm correlates with longer device lifespan *in vivo* and to determine the optimal frequency and duration of vibration application to maximize patient compliance. An IRB approved clinical trial in our health system is currently enrolling patients to further evaluate this.

This novel approach to TEP maintenance demonstrates promise in reducing the frustration of TEP failure and the cost of frequent replacement by laryngectomees.

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