

Porphyromonas Gingivalis Response to Ultrasonication

Kamineni Srinath^{1,*}, Dhawan Vikas¹, Huang, Chifu²

¹Department of Orthopaedics and Sports Medicine, Elbow Shoulder Research Center, University of Kentucky, Lexington, KY 40536

²Center for Oral Health Research, College of Dentistry; College of Dentistry University of Kentucky, Lexington, Kentucky 40503

Abstract

Introduction: Ultrasound technology has previously been applied for cataract removal and tennis elbow treatment. Recent data supports the use of ultrasonic debridement in the treatment of diabetic foot ulcers. No data is available concerning the potential antibacterial properties of a clinical grade, lower energy ultrasound probe. We investigated the effect of a ultrasonic probe with respect to *P gingivalis* bacterial viability.

Methods: A Tenex Tx1 probe with standard settings for clinical use was used for this study. A Gram negative (*Porphyromonas gingivalis*) bacteria, known for its pathological activity, was investigated. The bacteria was cultured in an anaerobic broth, re-suspended to achieve a consistent bacterial count, and 5ml of this re-suspension was placed in a test tube for testing. Each tube was sonicated with the Tx1 probe for varying lengths of time (10, 30, 60, 120 seconds). The sonicated was diluted and plated on blood-agar plates, followed by incubation for 48 hours at 37°C in an anaerobic growth chamber. The number of colony forming units were counted, on each plate and the anti-bacterial effect was calculated. A one way analysis of variance was performed for statistical analysis.

Results: A significant time-dependent antibacterial effect was demonstrated with sonication. When comparing the kill rate between the control and 120 seconds of sonication *P Gingivalis* had a 64% kill rate. This was the only statistically significant time comparison achieved, although the trend for all the time intervals was a reduction in the colony forming unit counts.

Conclusion: This study demonstrates that a clinically available ultrasonic probe (Tenex Tx1) has an antibacterial effect against the gram negative anaerobic bacterial species *P gingivalis*. Complete deactivation was not achieved, and there was a variation in effect dependent on the time of active sonication, with greater sonication times leading to greater kill rates. This data may partially help to explain the ability for ultrasonic debridement to result in the healing of long standing diabetic ulcers, that have been recalcitrant to other forms of treatment.

Corresponding author: Srinath Kamineni, Department of Orthopaedics and Sports Medicine, Elbow Shoulder Research Center, University of Kentucky, 740 South Limestone, Lexington KY 40536, USA, phone: +1 8598067703

Keywords: P Gingivalis

Received: Feb 10, 2019

Accepted: Mar 04, 2019

Published: Mar 07, 2019

Editor: Shivanthi Samarasinghe, De Montfort University, United Kingdom.

Introduction

Ultrasonic energy is known to be a part of the sound energy spectrum that agitates particles that come into contact with it. Many uses of ultrasonic energy have been documented, including the food industry, dental industry, and the medical industry.

Within the healthcare industry ultrasonic energy is used for different purposes, based on its frequency. For instance removing a cataract with an ultrasonic probe requires a different frequency to visualizing unborn foetus in a mother's womb using an diagnostic ultrasound machine, that in turn requires a different frequency when cutting and melting bone cement [1]. Recent innovations in the application of ultrasonic technology has included the ablation of local, non-metastasized, tumors [2, 3], and the treatment of recalcitrant diabetic foot ulcers, although the pathophysiological mechanism is unclear [4]. While there are potential possibilities for why ultrasonic debridement of a diabetic foot ulcer can be successful, an anti-bacterial mechanism is one such hypothesis, since there is significant data concerning the polymicrobial colonization of such ulcers. There is significant data available regarding aerobic and gram-positive bacteria. However, there is much less data available regarding gram-negative anaerobic bacilli, a category of bacteria that are difficult to eradicate in diseases such as gingivitis and diabetic foot ulcers [5]. We hypothesised that a possible mechanism for the recently observed success of ultrasonic debridement in diabetic foot ulcer treatment, was in part due to the antibacterial effect on *Porphyromonas gingivalis*, a gram-negative anaerobic bacillus, by the ultrasonic probe.

Materials and Methods

Bacterial Cultivation

Oral microbial species *Porphyromonas gingivalis* (ATCC 33277) was purchased from the American Type Culture Collection (Manassas, VA) [6, 7]. The anaerobe Broth was purchased from Oxoid Ltd. (Cambridge, UK). The bacteria was cultured under the growth conditions of 37°C in Plas-Labs anaerobic chamber with 85% N₂, 10% H₂, and 5% CO₂ (Lansing, MI).

Antibacterial Assay

Five millilitres of the bacterial culture was placed in a test tube for testing. Testing comprised of sonication using a TX1 ultrasonic probe (Tenex™, Lake Forest, CA), and was performed with the probe tip submerged in the bacterial suspension, without touching the test-tube. A normal function of this ultrasonic debridement system is to irrigate saline while that debrider is sonicating. In order to prevent dilution of our bacterial solution, this function was turned off. In order to ensure the broth was not heated by the ultrasonic energy, beyond an acceptable level, a thermometer was placed in the broth throughout the testing protocol, and it was ensure the temperature did not exceed 37.5°. Each sample was tested in triplicate for various times (10, 30, 60, 120 seconds). Following the sonication, 3 µl of the solution was diluted 10⁵ times and plated to blood agar plates (Remel®). The plates was incubated anaerobically, the maximal conditions for *P. gingivalis*, for 48 hours at 37°C. The colony forming units (CFU) were counted for each plate, for the entirety of the plate and not a particular quadrant or sector [6, 7].

Statistical Analysis

A one-way ANOVA with multiple comparisons was used for statistical analysis of the data. Multiple groups were compared using Turkey's post hoc adjustment, with SPSS v15 (IBM; Armonk, New York, USA). P values less than 0.05 were considered statistical significant.

Results

A time dependent antibacterial effect was evident with sonication, (Table 1). None of the times of testing completely denatured the bacterial colonies. The greatest anti-bacterial effect (the greatest reduction of colony forming units) was observed at 120 seconds, while lesser effects were observed at 10, 30, and 60 seconds. Sonication was effective in reducing the CFU counts in the G-negative *P. gingivalis*, bacteria (Table 2), when compared to the control at 120 seconds.

When comparing the kill rate between the control and 120 seconds of sonication, *P. Gingivalis* had a 64% kill rate (Figure 1). When comparing control to all of the time intervals tested, *P. Gingivalis* was only significant at 120 seconds, with nonsignificant results for

Table 1. Time effect of sonication the growth of P Gingivalis analyzed by microplating. Shown are the colony counts.

	PG
Control	840±26
10"	680±11
30"	572±13
60"	400±11
120"	304±8

Table 2. Statistical analysis of the effect of Tenex Sonication at various times.

	PG
0-120	0.0213
0-60	0.0288
0-30	0.3446
0-10	0.4277

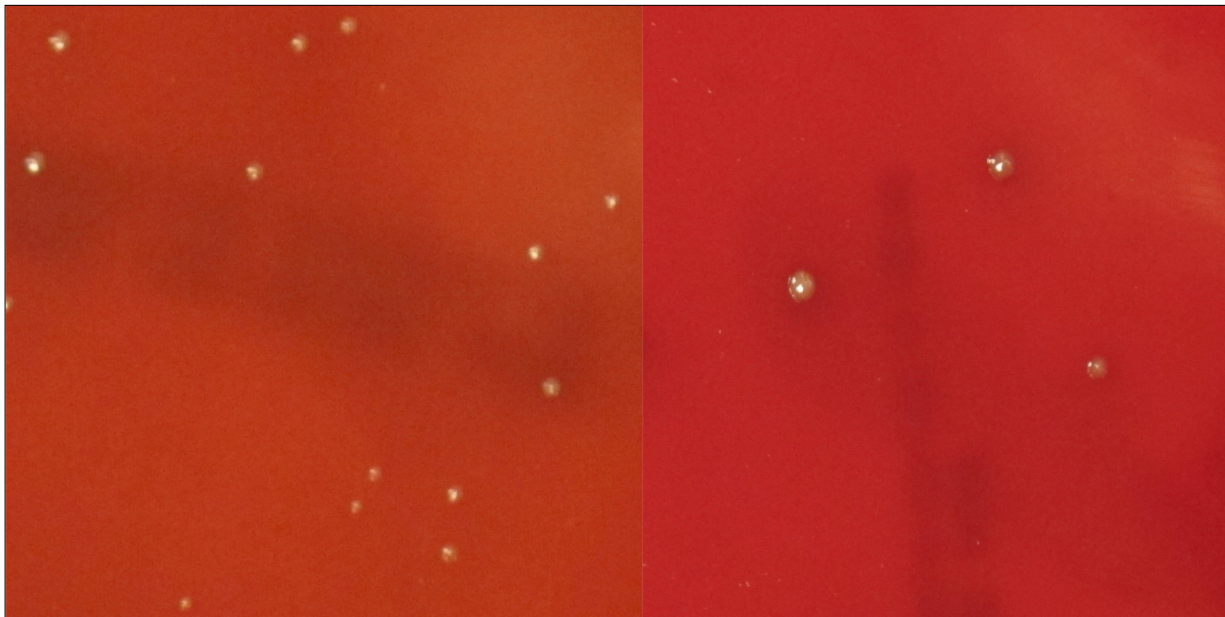


Figure 1. Porphyromonas Gingivalis; Culture plates (a) Control, (b) 120 seconds

the lesser time intervals.

Discussion

Ultrasonic energy has been demonstrated in many studies to have the capacity to neutralise bacteria, by disrupting cell walls, among other mechanisms of action. Ultrasonic inactivation of bacteria was first

reported in 1920s with the mechanism of inactivation investigations commencing in 1960s [1, 2] . Currently, there are many possibilities regarding the mechanism of antibacterial effect of ultrasonic energy. One such mechanism is believed to be the creation of cavities within the bacteria due to the acoustic energy, thereby directly creating a mechanical disruptive effect, and

damaging free radicals. High energy ultrasound is well known for its disruptive behavior towards bacterial structure, but lower energy ultrasound is poorly understood.

For instance, some reports showed that gram-negative bacteria was more sensitive to ultrasonic inactivation than gram-positive bacteria, while other researchers reported no significant relationship between the gram-status of bacteria and ultrasonic inactivation [1, 2].

This study demonstrates that a clinically available Tenex ultrasonic probe has an antibacterial effect against a particular gram negative, anaerobic bacillus, *P. gingivalis*. Complete disruption was not achieved, but there was a variation in the effect based the time of active sonication, with greater sonication times leading to greater kill rates. Although there was not statistical significance all of the time intervals tested, the trend was very clear an statistical significance was achieved at 120 seconds. This data helps to explain the ability for ultrasonic energy to result in dramatic healing responses of recalcitrant diabetic ulcers due to the reduction in the viable *P. gingivalis* load within the lesion [2, 4].

Limitations of the study included the use of a single bacterial species, and a small number of trials. Further studies are needed to better understand this technology with respect to a wider range of bacteria.

In conclusion, the Tenex Tx1 probe is effective at reducing the viable number of colony forming units in a time dependent manner, for *P. gingivalis*. While it was unable to achieve complete elimination, it was effective at decreasing *P. gingivalis* by 64%, with sonication of 120 seconds. These results are promising for the efficacy of procedures with *P. gingivalis* colonisation, notably diabetic foot ulcers, and possibly gingivitis an periodontal disease, when debrided with lower energy ultrasonic probes.

Acknowledgement

This work was not supported by any grants or donations.

References

1. Shengpu Gao, Gillian D. Lewis, Muthupandian Ashokkumar, Yacine Hemar. Inactivation of

microorganisms by low-frequency high-power ultrasound: 1. Effect of growth phase and capsule properties of the bacteria. *Ultrasonics Sonochemistry*, Volume 21, Issue 1, January 2014, Pages 446-453.

2. Guoping Huang, Suwan Chen, Chunhua Dai, Ling Sun, Wenli Sun. Yingxiu Tang, Feng Xiong, Ronghai He, Haile Ma. Effects of ultrasound on microbial growth and enzyme activity. *Ultrasonics Sonochemistry*, Volume 37, July 2017, Pages 144-149.
3. N. K. Chammas, 1 R. L. R. Hill, 2 and M. E. Edmonds. Increased Mortality in Diabetic Foot Ulcer Patients: The Significance of Ulcer Type. *Journal of Diabetes Research*, 2016.
4. Anamika Bajpai, Sumati Nadkarni, Michael Neidrauer, Michael S. Weingarten, Peter A. Lewin, Kara L. Spiller. Effects of Non-thermal, Non-cavitational Ultrasound Exposure on Human Diabetic Ulcer Healing and Inflammatory Gene Expression in a Pilot Study. *Ultrasound in Medicine & Biology*, Volume 44, Issue 9, September 2018, Pages 2043-2049.
5. Rafiei M, Kiani F, Sayehmiri K, Sayehmiri F, Tavirani M, Dousti M, Sheikhi A. Prevalence of Anaerobic Bacteria (*P.gingivalis*) as Major Microbial Agent in the Incidence Periodontal Diseases by Meta-analysis. *J Dent (Shiraz)*. 2018 Sep;19(3):232-242
6. Huang CB, Ebersole JL. A novel bioactivity of omega -3 polyunsaturated fatty acids and their ester derivatives. *Molecular Oral Microbiology*. 2010 Feb;25(1):75-80.
7. Huang CB, George B, Ebersole JL. Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. *Archives of Oral Biology*. 2010 Aug;55(8):555-60.