JAPMA Article In Press
This article has been reviewed and accepted for publication. Please note that it has not been copyedited, proofread, or typeset and is not a final version.

ORIGINAL ARTICLE

Optimization of Ultrasound-Mediated Drug Delivery for Treatment of Onychomycosis

Alina Kline-Schoder, BS*

Zung Le, DPM†

Liam Sweeney*

Vesna Zderic, PhD*

*Department of Biomedical Engineering, The George Washington University, Washington, DC.

†Department of Podiatry, The George Washington University Medical Faculty Associates, Washington, DC.

Corresponding author: Alina Kline-Schoder, BS, Department of Biomedical Engineering, 800 22nd Street NW, Washington, DC 20052. (E-mail: alinaks@gwu.edu)

Background: Onychomycosis is a fungal nail disorder that does not have a successful cure due to the poor permeability of topical anti-fungal drugs through the nail. This study utilizes ultrasound to increase the permeability of the nail to the topical drugs currently used in clinic. The first aim of this study was to optimize ultrasonic parameters within the temperature increase limits set by the American Institute of Ultrasound in Medicine (AIUM) and the British Medical Ultrasound Society (BMUS). The second aim of the study was to evaluate the optimized parameters for a cause of action of either cavitation (the creation of micrometer pores in the nail barrier) or acoustic streaming (a steady fluid motion which may help push the drug through the nail).

Methods: Porcine and human nails are used in the five studies. PZFlex Modeling Software is used to model the temperature increase in the toe as a result of ultrasonic application and these results were used to develop the three parameters tested throughout the rest of the studies. The three parameters tested were 1 min of continuous ultrasonic application, 3 min of 50% ultrasonic application and 5 min of 50% ultrasonic application. In order to address the second aim of our research work, these three parameters were tested for the presence of streaming and cavitation.

Results: At the three tested parameters, the most permeation of the nail occurs with 1 min of continuous application of ultrasound to the nail. It was also found that there was limited cavitation and significant streaming at all three parameters. This suggests that streaming may be the main mechanism-of-action in ultrasound-mediated drug delivery through the nail.

Conclusions: The parameter of 1 min of continuous ultrasonic testing will continue to be employed as the testing is moved to a rabbit model of onychomycosis.

Onychomycosis is a fungal nail disorder that causes nail thickening, discoloration and brittleness.[1] As a result of thickened nails, patients with onychomycosis experience discomfort and pain with normal nail use.[2] Notably, mycotic nails have been shown to have a negative psychosocial impact, particularly causing embarrassment and stigmatization.[3] If left untreated, onychomycosis increases the risk of severe infection, particularly in those with diabetes who are at risk of developing cellulitis, ulcers, and gangerene.[4]

Currently three main treatment plans are prescribed in clinic for onychomycosis treatment – an oral drug (Terbinafine, Itracanozole, or Fluconazole), a topical drug (Eficanozole, Ciclopirox, or Tavaborole) or a combination therapy of both an oral and topical drug.[5]

Terbinafine is the most commonly prescribed oral drug. However, according to 48 randomized studies, Terbinafine has a clinical cure rate of only 37% and the drug is associated with rare (2.5 in 100,000 patients), but dangerous side effects including elevated liver enzyme tests that can indicate liver damage and hepatitis.[5,6]

Although several topical drugs are currently approved for clinical use, the most popularly prescribed drug is Efinaconazole. Efinaconazole has been shown to be more effective than its main competitor, Ciclopirox.[7] In treatment, Ciclopirox or Efinaconazole is applied directly to the top of the nail and causes negligible side effects.[8] However, due to binding between the keratin in the nail and either drug, the anti-fungal drug progresses slowly and incompletely through the nail.[9] As a result, Efinaconazole has a cure rate of only 50% after 72 weeks of application and Ciclopirox has a cure rate of only 36% after 6 months of application.[10,11]

Ultrasound-mediated drug delivery has successfully been used through the skin and the eyes. [12,13] The two main mechanisms of action in ultrasound-enhanced drug delivery are cavitation and streaming. Cavitation is the creation of micrometer-size pores in the barrier. There are two types of cavitation – stable cavitation and inertial cavitation. Stable cavitation is the expansion and contraction of bubbles that are already present in or near the barrier in response to ultrasound. Inertial cavitation is the large size change of short-lived bubbles that eventually collapse. [14] Streaming is a force caused by the reduction in the ultrasound waves due to their absorption and scattering. [15] Specifically, micro-streaming is the movement of fluid in a localized area. Streaming has been shown previously to be the cause of action in drug delivery through the skin, and cavitation has been shown to be the main mechanism in ultrasound-mediated drug delivery through the cornea. [12,13]

Previous studies published by our laboratory confirm the efficacy of using ultrasound to increase drug delivery through the nail using porcine nails and a drug-mimicking blue dye. These same studies tested a range of frequencies (400 kHZ – 1 MHz) and found that the most drug delivery occurs with a frequency of 1 MHz. [16] These same studies found that the temperature increase after 5 min of continuous application at a frequency of 1 MHz, and an intensity of 1 W/cm^2 was close to the safety regulations of the American Institute of Ultrasound in Medicine (AIUM) and the British Medical Ultrasound Society (BMUS). [16] The first aim of this study was to test the efficacy of ultrasound-mediated drug delivery through the nail while testing the drug actually used in clinic, Ciclopirox. The second aim of this study was to optimize ultrasonic parameters while staying within the temperature increase considered safe by both the AIUM and the BMUS. In order to do this, safety modeling experiments were used to guide the optimization of the ultrasonic parameters which were then tested *in vitro* with human nails. Finally, cavitation and streaming studies were performed in order to examine the ultrasonic mechanism of action in nail drug delivery.

Materials and Methods

Five distinct sets of ultrasonic experiments are presented in this experimental work, as explained by the Table 1. The first study is the Ciclopirox Diffusion Cell Study, which was performed in order to confirm the efficacy of ultrasound-mediated drug delivery in increasing the permeation of the nail to one of the topical drugs used in clinic today. The second study was the Pulsed Modeling Study, which was performed in order to develop the ultrasonic parameters that were used for the final three studies, which were performed *in vitro*. The first set of these *in vitro* studies, the Luminosity Experiments was performed to assess the permeation of the excised human nail at the ultrasound parameters found to be safe by the Pulsed Modeling Studies. The second set of these *in vitro* studies, the Cavitation

Experiment, was performed to assess the impact of cavitation on the permeation of the nail at the chosen parameters. The final set of *in vitro* studies, the Streaming Experiment, was performed to assess the impact of streaming on the permeation of the nail at the chosen parameters.

A. Ultrasound Parameters

The unfocused circular ultrasound (US) transducers used in all *in vitro* experiments had an active diameter of 15 mm and center frequencies of 400, 600, and 800 kHz and 1 MHz (Sonic Concepts, Inc, Bothell, WA). The US waveforms were developed by a function generator (Agilent Technolo- gies, Santa Clara, CA) and amplified to obtain a 50-dB gain by an amplifier (150A100B RF amplifier; Amplifier Research, Souderton, PA). The US used for all experiments was set to be 1 W/cm^2 at all parameters used.

B. Diffusion Cell Experiment Materials and Methods The Diffusion Cell Experiments quantified permeation through the entire nail using a Franz Diffusion Cell (Fig. 1). These experiments utilized porcine nails (Sioux-Preme Packing Company) and Ciclopirox. The diffusion cell (PermeGear, Hellertown, PA, USA) was fit with a custom-made nail and lid adaptor in order to prevent leakage of drug around the nail while still allowing the ultrasound transducer and 50 mL of dye to fit above the setup. The nail adaptor is explained in detail in our previous publication.[17]

This experiment utilized pig feet that were obtained from Sioux-Preme Packing Company (Sioux Center, IA). The nails were separated with a scalpel and razor before being stored at 10.6 ˚C until their use. The pieces of porcine nail (n=5) were cut to be approximately the size of a human nail (1 x 1 x 0.1 cm).

In these experiments, the donor compartment was filled with Ciclopirox and the receiving compartment was filled with ethanol due to the hydrophobicity of Ciclopirox. [18] Throughout the

experiments, the entire diffusion cell was placed in a water bath at 37° C and the receiving compartment was stirred using a magnetic stirring bar that spun at 450 RPM. The nail was sonicated from a set distance of 85 mm and an intensity of 1 +/- 0.1 W/cm^2.[17] The nail was sonicated at either 400 kHz, 600 kHz, 800 kHz and 1 MHz for five minutes. After each experiment, 2 mL of solution was collected from the receiving compartment of the diffusion cell and a spectrophotometer (UVmini-1240; Shimadzu, Columbia, MD) was used to measure its absorption with ethanol as the base. Absorbance was measured at 360 nm. This number was found by performing an initial calibration curve on the Ciclopirox. Two serial dilutions totaling 26 measurements of drug in ethanol were also performed at this wavelength to develop an equation to convert from absorption measurement to dilution. This methodology is explained in detail in previous literature.[17]

C. Pulsed Modeling Studies Materials and Methods

The Pulsed Modeling Studies were performed with the intention of optimizing ultrasound parameters within the temperature increase guidelines of the British Medical Ultrasound Society and the American Institute of Ultrasound in Medicine.[19,20] PZFlex, an explicit time-domain modeling software (Weidlinger Associates, Mountain View, CA, USA) was utilized for the entirety of this experiment. The model used had symmetric axes and absorbing boundaries and its convergence was successfully checked using 11 simulations, as was reported in the laboratory's previous study.[17] For the purpose of this experiment, the human toe was modeled as being 2-dimmensional, straight and rectangular. In the human body, the toe is relatively symmetrical, so this representation is realistic. However, the human toe is slightly curved, and this variation may have caused slightly simulated values that are slightly higher than are actually expected.[21]

The nail was modeled using the five layers of the toe as defined by thickness in Table 2. Structure thickness was estimated using literature and due to PZFlex allowances was rounded to the nearest 0.5 mm.

For the frequency of 1 MHz and the transducer diameter of 20 mm, the transition point from near field to far field (DFF) was calculated to be 55 mm for the applied intensity of 1 W/cm^2.[14] Experiments were performed in the near-field at a distance of 31 mm. The distance was changed from the Ciclopirox Diffusion Cell Studies in an effort to make the work more clinically applicable. The times of application that were tested were 1 min, 3 min, and 5 min. In previous studies, the temperature increase due to ultrasound was found to be over the safety limit, so pulsing was used.[17] The pulsing tested ranged from 3% to 100%.

The results of the PZFlex study provided three sets of parameters – one at each time of application (1 min, 3 min, and 5 min) to be tested in the two other *in vitro* studies.

D. Luminosity Materials & Methods

The two *in vitro* experiments utilized planar ultrasound transducers, an intensity of 1 W/cm^2, and a frequency of 1 MHz. The unfocused circular US transducer used in all experiments had an active diameter of 20 mm and a center frequency of 1 MHz. The *in vitro* experiments utilized onychomycotic human nails that were excised (under an approved IRB protocol) as a part of a standard patient treatment.

The Luminosity Experiments quantified nail permeation by measuring the amount of drug-mimicking blue dye (FD&C Blue No. 1 – Brilliant Blue FCF, E133) that entered the nail as a result of sonication (Fig. 2).

Pieces of diseased human nail (0.1 x 0.1 x 1 cm^3) were placed in a 100 mL beaker that was filled with the dye, 31 mm below the transducer. Nails were then sonicated at one of the three parameters found in the PZFlex Modeling Experiments given the pulsing allowances of the physio-ultrasound (Sonicator 740, Mettler Electronics Corp, Anaheim, CA, USA) device (n=5). The beaker was held at 37° C using a water bath (Thermo Haake DC10-P21, Fisher Scientific, Waltham, MA, USA). The blue dye used a molecular weight of 792.84 g/mol and was polar. For comparison, Ciclopirox has a molecular weight of 207.27 g/mol and is nonpolar and Eficanozole has a molecular weight of 348.39 g/mol and is also nonpolar. However, all three molecules are considered small molecules, and for small molecules, the permeability varies inversely with molecular weight, meaning that Ciclopirox and Eficanozole would likely have greater permeability than the blue dye used in the experiments.[22]

After treatment, the nails were cut and photographed (iPhone 6, Apple Inc, Cupertino, CA, USA). MATLAB was used to crop the image so that it only contained the cross section of the nail and then to analyze the average brightness – and therefore permeation of dye through the nails' cross section. MATLAB quantifies brightness using the brightness constant, b (b of 250 is a white image, and b of 0 is a black image). The brightness constant was used to develop the luminosity value v that increases with an increase in nail permeation (1).

$$10/b = v$$

An unequal variance two-tailed student's t-test was used to compare the luminosity value with the sham experiments.

E. Cavitation Experiment Materials and Methods

The Cavitation Experiments (Fig. 3) quantified both inertial and stable cavitation at the three different parameters used throughout this work in an attempt to understand the mechanism of action of the

ultrasound-mediated drug delivery for onychomycosis. Diseased human nails were used (n=8). The Cavitation Experiments were performed both with (Fig. 3a), and without (Fig. 3b) a nail in order to quantify cavitation. An acoustic absorber was placed in a water bath at 37˚C, and then sonicated from 31 mm at the parameters of the study. Passive Cavitation Detection (PCD) was then used to characterize the system. A PCD is a type of hydrophone that specifically receives the range of frequencies in which cavitation-induced acoustic signals occur. [3].

This study utilized a single-element PCD transducer (bandwidth of 2.8–4.2 MHz; ISO304HP, CTS Valpey, Hopkinton, MA, USA). The PCD was aimed at the absorber (with or without the nail) so that it intersected the path of the ultrasound. The signals obtained from the PCD were collected by a spectrum analyzer (MDO3024, Tektronix, Arlington, VA, USA), and the data was saved for analysis in MATLAB. Stable cavitation was quantified by identifying both subharmonics and ultra-harmonics of 1 MHz, because emission of both of these harmonics occurs with stable cavitation .[14] Inertial cavitation was quantified by analyzing the broadband noise across the frequency spectrum of the signal because inertial cavitation causes chaotic oscillation.[24]

Broadband noise was quantified by fitting the frequency spectrum with an eighth-order polynomial in order to decrease the weight of the signal's harmonic peaks. The signal was then integrated across the bandwidth in order to quantify the broadband noise, in a method reported in previous studies.[23]

F. Streaming Experiment Materials and Methods

The Streaming Experiment setup is shown in Fig. 4 and was performed very similarly to the Luminosity Experiment. Pieces of diseased human nail (n=8) were placed in a 100 mL beaker that was filled with saline solution, 31 mm below the transducer. Nails were then sonicated at one of the three parameters

specified by the modeling experiment. The beaker was held at 37°C using a water bath (Thermo Haake DC10-P21, Fisher Scientific, Waltham, MA, USA).

After this first sonication, nails were transferred to a second 100 mL beaker that was filled with the same blue dye solution that was used in the Luminosity Experiments. For this round of treatment, nails were placed in blue dye for the same length of time as the original experiment but without any ultrasonic application.[25]

After treatment, the nails were assessed in the same manner as the nails from the Luminosity Experiment. These nails were considered the Experimental Nails (US + Saline + Dye) of the study. Nails were compared to the Negative Control Group (Dye) from the luminosity experiment and the Positive Control Group (US + Dye) from the luminosity experiment using an unequal variance two-tailed student's t-test (n=5).

Results

A. Diffusion Cell Results

In the Ciclopirox Diffusion Cell Experiment, the 800 kHz and 1 MHz ultrasound tests were statistically significant ($p<0.05$) as compared to sham treatments. The 800 kHz experiment found an increase in nail permeation of 29% over control ($p<0.05$) and the 1 MHz experiment found an increase of 425% ($p<0.05$) as compared to the control. The increase in the 600 kHz test was 3% and the increase in the 400 kHz experiment was 15%. These results are displayed in Fig. 5.

B. PZFlex Results

The PZFlex results are shown in Fig. 6. Using the American Institute of Ultrasound in Medicine (AIUM) standards for 5 minutes of ultrasonic application, a temperature increase of 4.5°C is considered safe, but

according to the British Medical Ultrasound Society's (BMUS) safety considerations, a temperature increase of only 3˚C is considered safe. According to our results, a duty cycle of 50% must be used in order to obey both safety standards (temperature increase of 2.5˚C). [19,20]

The AIUM standards for 3 minutes of ultrasonic application consider a temperature increase of 5.25˚C to be safe, but the BMUS only considers a temperature increase of 3˚C to be safe. According to these limits, a duty cycle of 65% (temperature increase of 2.9˚C) must be used in order to remain below both safety levels.[19,20]

For 1 minute of ultrasound exposure, the AIUM considers a temperature increase of 6˚C to be safe, and the BMUS considers a temperature increase of 4˚C to be safe. According to these standards, a 100% duty cycle can be used to remain below both safety levels (temperature increase of 3.91˚C).[19,20]

C. Luminosity Results

The luminosity results (Fig. 7) were statistically significant ($p<0.005$) at the three parameters that were tested. However, no statistical significance was found when the three experimental groups were compared to each other. The increase in nail permeability was found to be 74.4% when the nail was treated for 1 min of continuous ultrasound, the increase in nail permeability was found to be 39.1% when the nail was treated for 3 min and 50% pulsing, and the increase in nail permeability was found to be 19.3% when the nail was treated for 5 min and 50% pulsing (n=5).

The increase in nail permeability simply increased with time in the sham studies, but this effect was not noticed in the experimental groups. In the experimental groups, the 1 min/100% Group had the highest average luminosity value of 0.112, and the 3 min/50% Group had the lowest luminosity value of 0.100, with the 5 min/50% Group in the middle with a luminosity value of 0.103.

D. Cavitation Results

The Cavitation Results were broken into two parts – the Stable Cavitation Results (Fig. 8) and the Inertial Cavitation Results (Fig. 9).

The Stable Cavitation Results highlight the subharmonic and ultraharmonic in red and highlight the harmonics in yellow. The subharmonic for all experiments is located at 500 kHz and the ultra-harmonic is located at 1.5 MHz. The subharmonic and ultraharmonic are small in all of the samples. However, both the subharmonic and ultra-harmonic are larger in the studies performed with the nail than in the studies performed without the nail. This is particularly noticeable when directly comparing the nail and no-nail studies that were performed at 5min/50%.

The Inertial Cavitation Results are shown in Fig. 9. Fig. 9 quantifies the broadband noise at each of the tested parameters by integrating the frequency spectrum at that parameter. The integral value is entitled the Inertial Cavitation Value. The Inertial Cavitation Values are systematically higher for the nail experiments than the no-nail experiments at the same parameters, and this data is statistically significant at all three parameters ($p<0.05$). The highest Inertial Cavitation Value for the nail experiments was found to occur in the 3 min/50% Experiment. The 1 min/100% and 5 min/50% experiments had similar results that were slightly lower. However, the differences between the individual nail experiments were not found to be statistically significant.

The no-nail experiments show a different trend. The highest Inertial Cavitation Value of these experiments was found in the 5 min/50% experiment and the lowest Inertial Cavitation Value of these experiments was found in the 1 min/100% continuous experiment. The no-nail experiments were found to be statistically significant from one another, with the sole exception of the difference between the 3 min/50% and the 5 min/100% no-nail experiments.

E. Streaming Results

The streaming results are presented in Fig. 10. The average luminosity value of the control nails (exposed to ultrasound and dye) is found to be significantly higher than the experimental (US + Saline + Dye) luminosity value at all frequencies ($p<0.05$). There was no statistical significance between the luminosity values of the Experimental and Negative Control (Dye) groups. Additionally, there was no statistical significance between the three experimental groups. The nail exposed to 3 min of ultrasound at 50% had the lowest luminosity value as compared to both the 5 min of ultrasound at 50% pulsing and the 1 min of continuous ultrasound. The 3 min of ultrasound at 50% of pulsing was also the nail that found the lowest diffusion in the Luminosity Experiments.

Discussion

In the Ciclopirox Diffusion Cell Experiments, the 800 kHz and 1 MHz treatments were found to be statistically significant as compared to the sham treatment, with more permeation in the 1 MHz treatment. This Ciclopirox experiment confirmed the efficacy of ultrasound-mediated drug delivery in the *in vitro* nail. This experiment also increased the evidence that 1 MHz may be the optimal frequency for increasing delivery through the nail.

In the PZFlex simulations, the change in temperature is due to the balance of heat loss and heat gain in the tissues exposed to ultrasound.[26] Heat gain is determined by the absorption characteristics of each tissue, whereas the composition and vascularity of the tissue determines the heat loss. The simulations found maximum temperature increases in the bone. The bone has the largest acoustic attenuation constant, and higher acoustic attenuation correlates to more heat absorption, making the results consistent with the expected results.[26]

To be a complete model of the human toe the model requires perfusion. Perfusion is recorded to decrease the temperature by 10%, which means that the results found in this study should be 10% higher than those found in a living model. [27] However, the PZFlex results are likely a good prediction of the temperature increase in the living model.

In the luminosity experiments, although the experiments performed at the three parameters were not found to be statistically different from one another, all three were found to be statistically significant from their relative sham experiments. This means that nail permeation occurred to a significant degree at each parameter tested. The most permeation as compared to the control occurred in the nails that were exposed to ultrasound for 1 min of continuous ultrasound, which suggests this is the best parameter to use in future experiments.

The experiments found that the stable cavitation was smaller in the studies performed with a nail as compared to the studies performed without a nail. This is the expected result, as the nail is a solid barrier, and solid barriers decrease stable cavitation.[28] Conversely, inertial cavitation was higher in the studies performed with a nail than in the studies performed without a nail, which was also the expected result.[29] Overall, very small amounts of cavitation were found across the experiment. This means that cavitation is unlikely the cause of nail permeation at the parameters used.

The streaming experiments indicate that streaming may have a large impact on the nail at all three parameters tested. The experimental nails (US + Saline) were found to have permeation comparable to the negative control (Dye), indicating that with the mechanical impact of streaming removed, the permeation becomes insignificant, so streaming is likely the main ultrasonic mechanism-of-action at all three tested parameters.

Previous studies performed by our laboratory demonstrated proof-of-concept through the porcine and canine nail.[17] [33] Ultrasound-mediated drug delivery through the cell membrane and the skin

has been shown to be related to cavitation.[30, 31, 32] The results found in this study suggest that cavitation may not have a large impact in ultrasound-mediated drug delivery through the nail. The results presented in this study suggest not only the optimal parameters for ultrasound-mediated delivery through the nail, but also the main mechanism-of-action at this parameter.

In addition to increasing permeation through the nail, ultrasound has been shown to decrease fungus through the creation of hydroxyl radicals. This mechanism may assist the delivery and will be tested in further studies. [34]

Additional further studies will continue to test the efficacy of ultrasound in increasing drug delivery of Ciclopirox and/or Eficanozole through the human nail and steps will be made towards using a rabbit model of onychomycosis.

Conclusions

Ultrasound presents a novel technique to increase drug delivery through the nail for improved treatment of onychomycosis. Our *in vitro* studies found the most compound (both drug-mimicking dye and drug) permeated the nail when 1 MHz ultrasound was used for treatment at 1 min of continuous application. The data suggest a possibility for ultrasound-mediated drug delivery to eventually make its way into clinical practice. Future *in vivo* experiments will focus on implementing a rabbit model of onychomycosis.

References

1. Hay RJ, Baran R: Onychomycosis: a proposed revision of the clinical classification. J Am Acad Dermatol. 65: 1219, 2011.
2. Turner RR, Testa MA: Measuring the impact of onychomycosis on patient quality of life. *Qual Life Res.* 9: 39, 2000.
3. Szepietowski JC, Reich A: Stigmatisation in onychomycosis patients: a population-based study. National Quality of Life in Dermatology Group. 52: 343, 2009.
4. Winston JA, Miller JA: Treatment of onychomycosis in diabetic patients. Clin Diabet. 24: 60, 2006.
5. Kreijkamp-Kaspers S, Hawke KL, van Driel ML: Oral Medications to Treat Toenail Fungal Infection. JAMA. 319: 397, 2018.
6. Vickers, AEM, Ulyanov, AV, Fisher, RL: Safety and tolerability of oral antifungal agents in the treatment of fungal nail disease: a proven reality. Therapeutics and Clinical Risk Management. 1:299, 2005.
7. Tachibana H, Kumagai N, Tatsumi Y: Fungicidal Activity in the Presence of Keratin as an Important Factor Contributing to In Vivo Efficacy: A Comparison of Efinaconazole, Tavaborole, and Ciclopirox. Journal of Fungi. 3: 58, 2017.
8. Rodriguez DA: Efinaconazole Topical Solution, 10%, for the Treatment of Mild and Moderate Toenail Onychomycosis. The Journal of Clinical and Aesthetic Dermatology. 8: 24, 2015.
9. De Berker D: Fungal nail disease. N Engl J Med. 360: 2108, 2009.
10. Pollak, R, Ilie, C: Long-Term Follow-up of Onychomycosis Patients Treated With Efinaconazole. Journal of Drugs in Dermatology. 16: 1269, 2017.

11. Gupta AK, Joseph WS: Ciclopirox 8% nail lacquer in the treatment of onychomycosis of the toenails in the United States. J Am Podiatr Med Assoc. 90: 495, 2009.
12. Tang H, Mitragotri S, Blankschtein D, Langer R: Theoretical description of transdermal transport of hydrophilic permeants: application to low-frequency sonophoresis. J Pharm Sci. 90: 545, 2001.
13. Oberli MA, Schoellhammer CM, Langer R, Blankschtein D: Ultrasound-enhanced transdermal delivery: recent advances and future. Ther Deliv. 5: 843, 2014.
14. Christensen DA. *Ultrasonic Bio-instrumentation.* Hoboken, NJ: John 41. Wiley & Sons; 1988.
15. Loh B, Hyun S, Ro P, Kleinstreuer C. Acoustic streaming induced by ultrasonic flexural vibrations and associated enhancement of convective heat transfer. J Acoust Soc Am. 111: 875, 2002.
16. Tang H, Mitragotri S, Blankschtein D, Langer R: Theoretical description of transdermal transport of hydrophilic permeants: application to low-frequency sonophoresis. J Pharm Sci. 90: 545, 2001.
17. Kline-Schoder, A, Le, Z, Zderic, V. Ultrasound-Enhanced Drug Delivery for Treatment of Onychomycosis. J Ultrasound Med. 14526: 10, 2017.
18. Garg, T, Rath, G, Goyal, AK. Comprehensive review on additives of topical dosage forms for drug delivery. Drug Delivery. 22: 969, 2014.
19. British Medical Ultrasound Society. Guidelines for the safe use of diagnostic ultrasound equipment. Ultrasound 18: 59, 2010.
20. American Institute of Ultrasound in Medicine. Statement on mam- malian biological effects of ultrasound in vivo. American Institute of Ultrasound in Medicine website; 2008. http://www.aium.org/resour- ces/statements.aspx.

21. Nell DM, Myers MR. Thermal effects generated by high-intensity focused ultrasound beams at normal incidence to a bone surface. J Acoust Soc Am. 127: 549, 2010.
22. Basini P, Kr DB, Sudip D, Subhas S. Nail drug delivery system: a review. J Adv Pharm Technol Res. 2: 101, 2012.
23. Suarez Castellanos I, Jeremic A, Cohen J, Zderic V. Ultrasound stimulation of insulin release from pancreatic beta cells as potential novel treatment for type 2 diabetes. Ultrasound Med Biol. 43: 1210, 2017.
24. Leighton TG. The acoustic bubble. London/San Diego: Academic Press; 1994.
25. Zderic, V. Ultrasound-Enhanced Ocular Drug Delivery. University of Washington. 2004
26. Barnett, SB, Rott, HD, Ter haar, GR, Ziskin, MC, Maeda, K. The sensitivity of biological tissue to ultrasound. Ultrasound Med Biol. 23: 805, 1997.
27. Horder MM, Barnett SB, Vella GJ, Edwards MJ, Wood AK. In vivo heating of the guinea-pig fetal brain by pulsed ultrasound and estimates of thermal index. Ultrasound Med Biol. 24: 1467, 1998.
28. Leighton TG. The acoustic bubble. London/San Diego: Academic Press; 1994.
29. Leighton TG. The acoustic bubble. London/San Diego: Academic Press; 1994.
30. Guzman, HR, Nguyen, DX, McNamara, AJ, Prausnitz, MR. Equilibrium loading of cells with macromolecules by ultrasound: Effects of molecular size and acoustic energy. J Pharm Sci. 91: 1693, 2002.
31. Zderic V, Clark JI, Martin RW, Vaezy S. Transcorneal drug delivery using 880 KHz ultrasound. Cornea. 23: 804, 2004a.
32. Zderic V, Clark JI, Vaezy S. Drug delivery into the eye with the use of ultrasound. J Ultrasound Med. 23: 804, 2004b.

33. Abadi D, Zderic V. Ultrasound-mediated nail drug delivery system. J Ultrasound Med. 30: 1723, 2011.

34. Iwasawa A, Saito K, Mokudai T, Kohno M, Ozawa T, Niwano Y. Fungicidal action of hydroxyl radicals generated by ultrasound in water. J Clin Biochem Nutr. 45: 204, 2009.

Table 1. Experimental Chart.

	Diffusion Cell	PZFlex Experiments	Luminosity Experiments	Mechanistic Studies		
				Cavitation		Streaming
				Stable	Inertial	
Type	*In vitro*	*In silico*	*In vitro*	*In vitro*	*In vitro*	*In vitro*
Nail	Porcine	N/A	Onychomycotic Human	Onychomycotic Human	Onychomycotic Human	Onychomycotic Human
Frequency (MHz)	0.4, 0.6, 0.8, 1	1	1	1	1	1
Exposure Time & Duty Cycle	5 min (100%)	A. 1 min (0-100%) B. 3 min (0-100%) C. 5 min (0-100%)	A. 1 min/100% B. 3 min/50% C. 5 min/50%	A. 1 min/100% B. 3 min/50% C. 5 min/50%	A. 1 min/100% B. 3 min/50% C. 5 min/50%	A. 1 min/100% B. 3 min/50% C. 5 min/50%

Table 2. Tissue Properties

Structure	Speed of sound (m/s)	Specifi Heat (J/kg/K)	Thermal Conductivity W/mK	Density (kg/cm^3)	Acoustic Attenuation (dB/cm/MHz)	Thickness (mm)
Nail	2549	1680	0.291	1270	1.83	0.6
Skin	1537	3391	0.293	1093	0.293	0.5
Subcutaneous Tissue	1477	2348	0.21	911	0.61	3.5
Bone	2405	2274	0.29	1330	4.07	5.1

Fig 1. Diffusion Cell: Ciclopirox diffusion cell experimental design. Receiving compartment was filled with ethanol and donor compartment was filled with Ciclopirox while magnetic spinner was spun at 450 RPM as nail was sonicated.

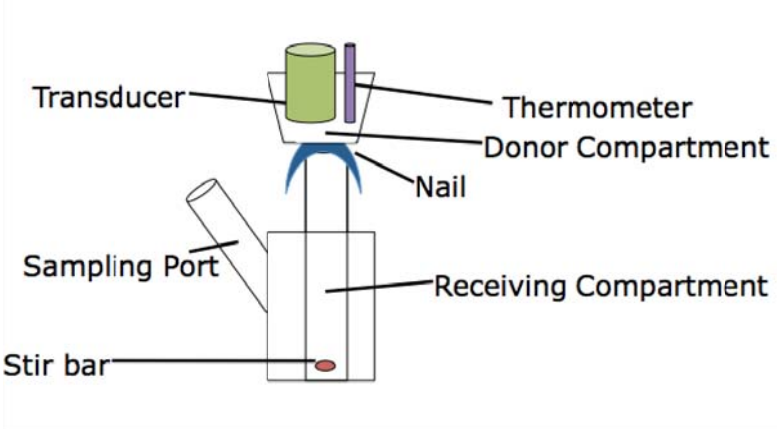

Fig 2. Luminosity Setup: Luminosity Experiment experimental design. Nail was sonicated in blue dye at set distance from the ultrasound transducer. Nail was placed on acoustic absorber.

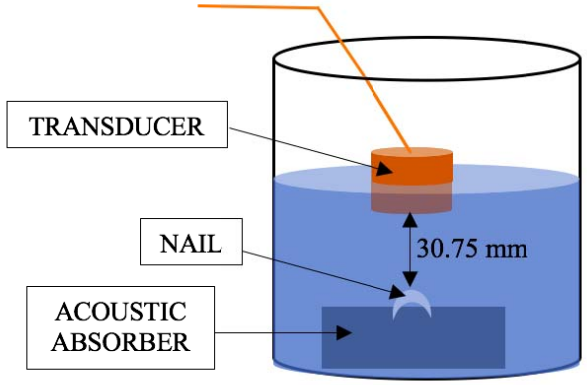

Fig 3. Cavitation Experimental Design A) With Nail B) Without Nail: Cavitation Experiment experimental design. Acoustic Absorber (+/- Nail) was placed in water bath at set distance from an ultrasound transducer. The PCD was set to intersect the path of ultrasound.

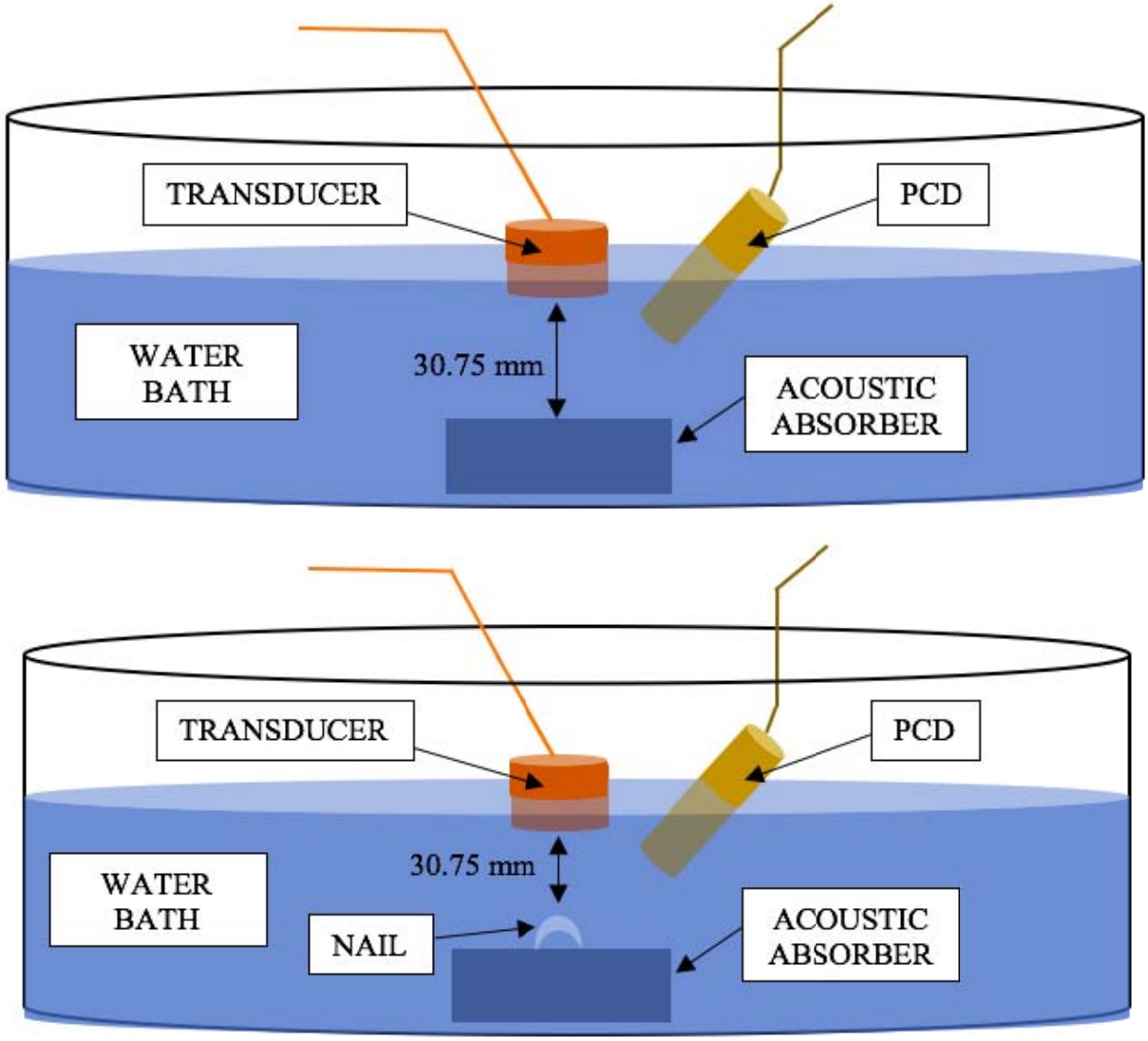

Fig 4. Streaming Setup: Streaming Experiment experimental setup. Nail was first placed in saline at set distance from ultrasound transducer. Nail was then moved into blue dye for set amount of time with no sonication.

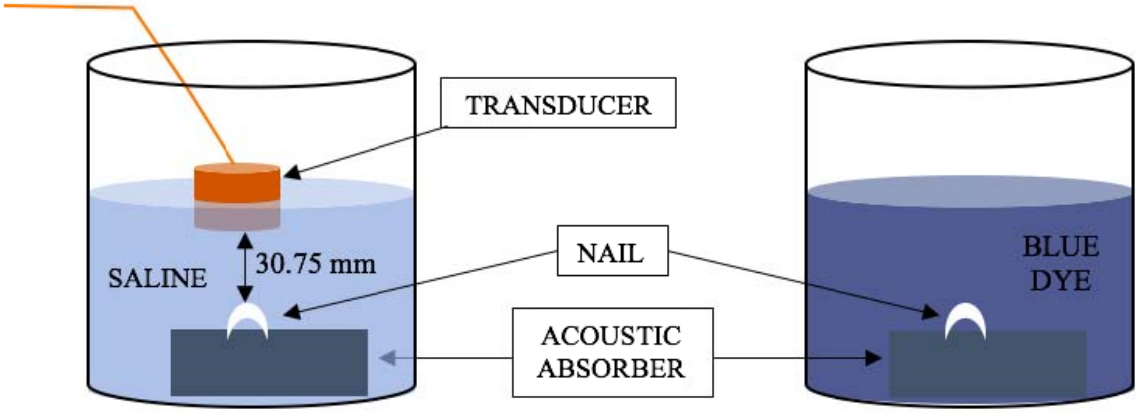

Fig 5. Diffusion Cell Results: Dilution of receiving compartment as function of ultrasonic frequency. Dilution was determined by performing spectrophotometry on the receiving compartment of the diffusion cell after treatment.

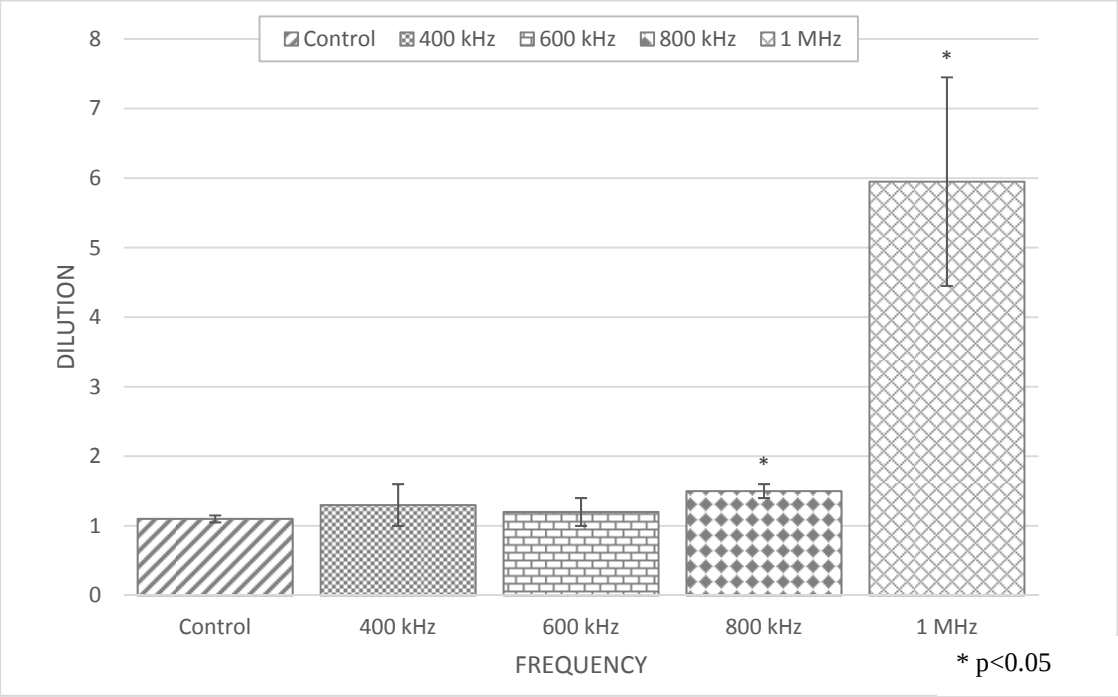

Fig 6. PZFlex Pulsing Results: Temperature increase in toe as a function of pulsing in 1 min, 3 min and 5 min of applied ultrasound. Temperature increase is compared to BMUS and AIUM limits, which are plotted in orange and gray, respectively.

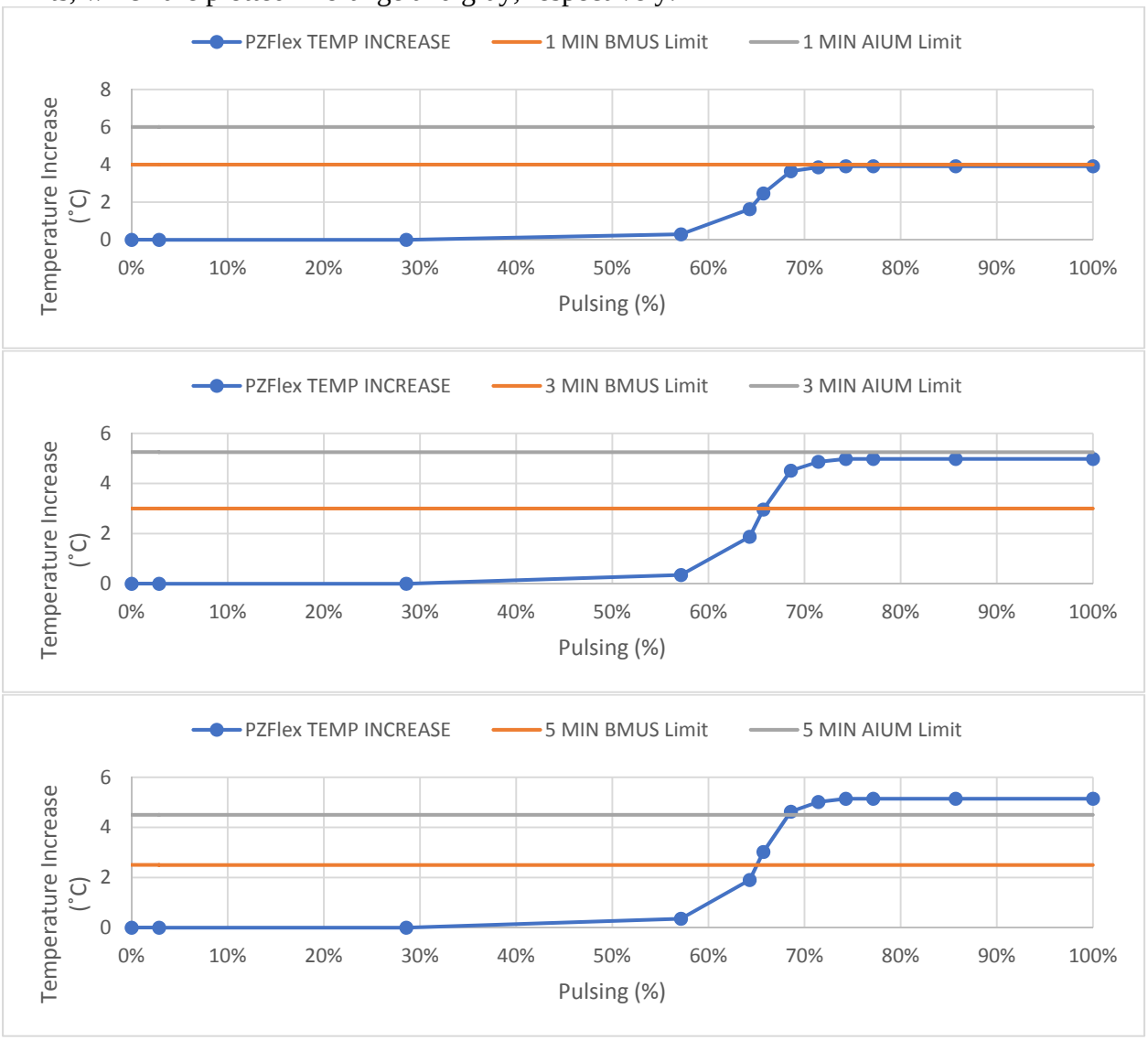

Fig 7. Luminosity Results: Luminosity value as a function of experimental parameter (both time and pulsing). Luminosity value was found by taking cross section of nail and finding average brightness and therefore permeation through the nail.

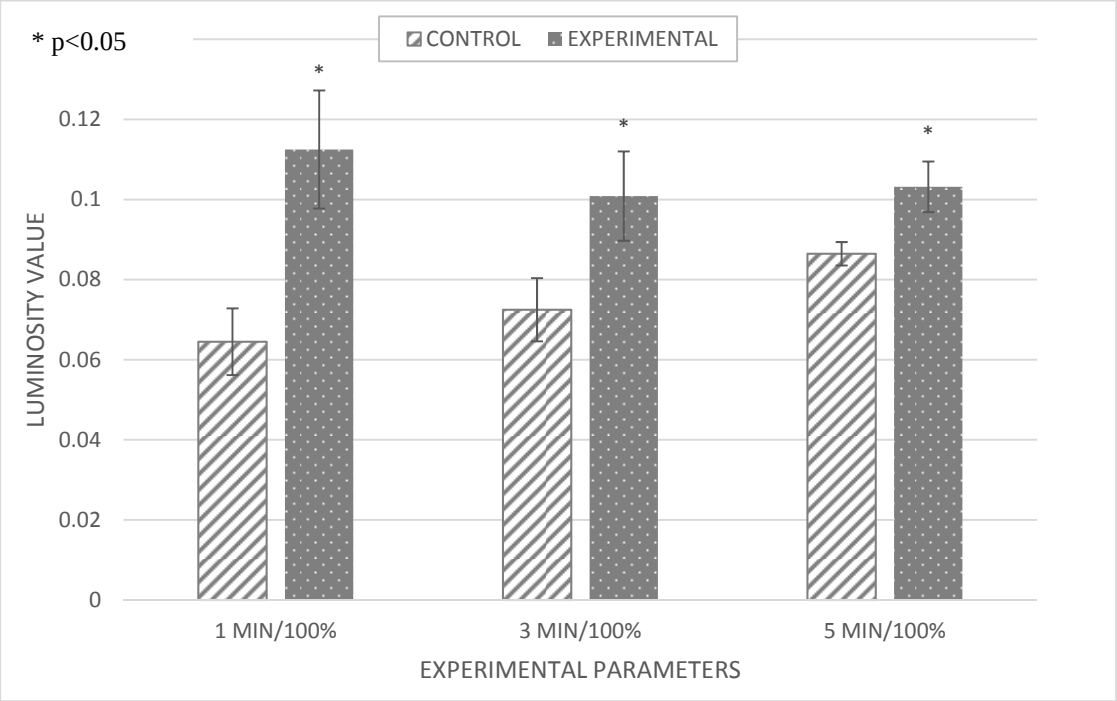

Fig 8. Stable Cavitation Results: Stable cavitation results. Plots show sub-harmonic, ultra-harmonic (red) and harmonics (green) of the frequency spectrums obtained for three parameters both with and without nail. Results were obtained using a PCD.

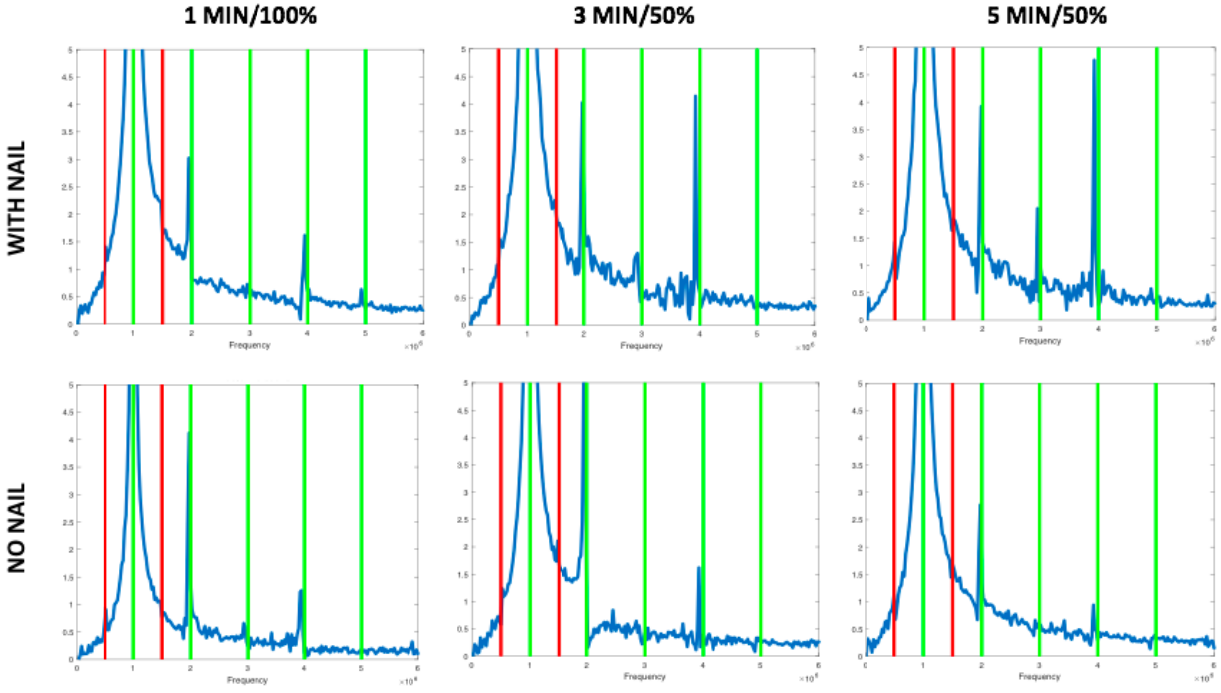

Fig 9. Inertial Cavitation Results: Inertial cavitation value as a function of experimental parameter (both time and pulsing). Inertial Cavitation Value was obtained by integrating the frequency spectrum found using a PCD at the various parameters.

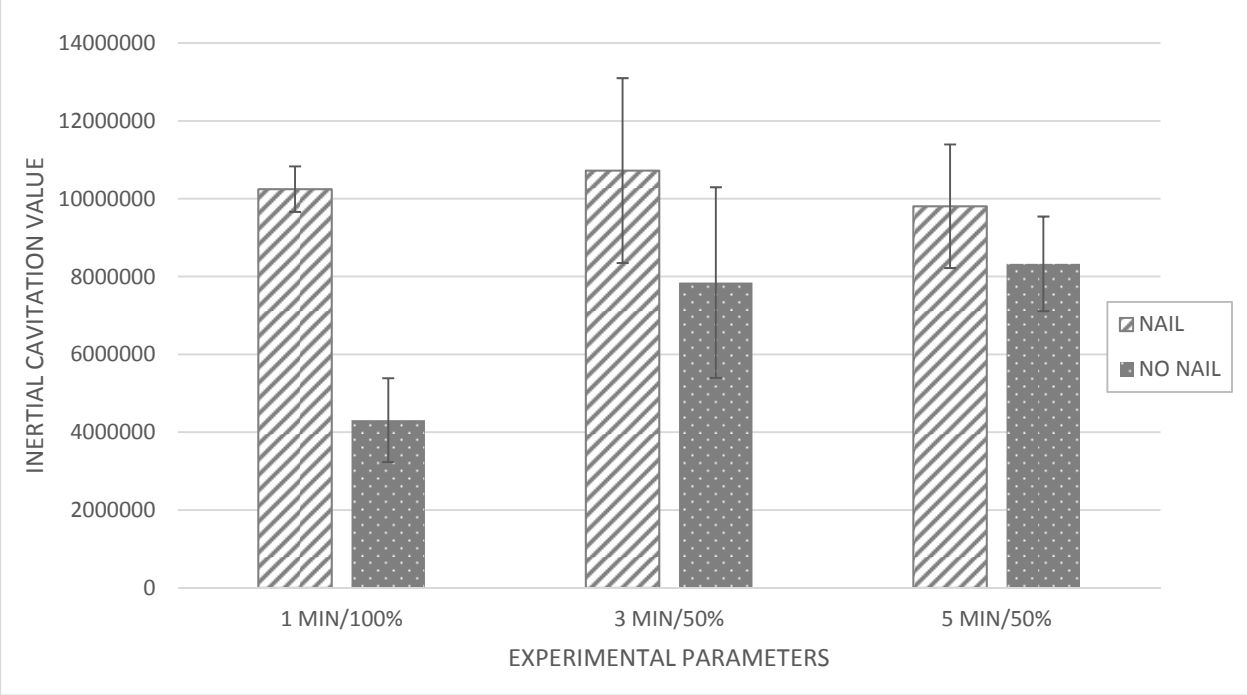

Fig 10. Streaming Results: Luminosity value as a function of experimental parameters. Luminosity value was obtained by taking cross section of treated nail and finding average brightness (and therefore permeation). Comparison of experimental group that eliminates streaming with positive control that has known nail permeation and negative control which has normal nail permeation. Statistical comparison was performed between the positive control and the experimental group.