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Externally Applied Vibration at 50 Hz Facilitates Dissolution of Blood Clots In-Vitro

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Abstract

Background: Localized Low Frequency Vibration (LLFV) in the low sonic range is utilized for disruption of clots by direct contact in catheter applications. However enhanced clot dissolution whereby an LLFV source is applied external from a clotted lumen (such as to resemble a non-invasive therapy) has not been studied.

Objective: To assess the effectiveness of low amplitude extra luminally applied 50 Hz LLFV in dissolution of 1 hr old clots immersed in Heparinized Saline.

Methods: One hr old blood clots were each placed within a 3 ml syringe filled with 1.5 ml's of Heparinized Saline. LLFV was then applied against the external surface of each syringe with gentle stroke amplitude (~ 0.5 mm), an intensity within an order of magnitude expected to reach a thrombosed vessel (such as a coronary, pulmonary, cerebral, or peripheral artery), given if a substantially stronger application where to be applied non-invasively across the artery's overlying soft tissue barrier.

Results: LLFV yielded statistically superior clot dissolution (25%) in comparison to the non vibrated controls (5%) ($p<0.0003$).

Conclusions: Transluminally applied LLFV (50 Hz) accelerates clot dissolution in vitro. Further study in this area in-vivo appears warranted.

Keywords: Vibration; Percussion; Thrombosis; Thrombolysis, Acute Myocardial Infarction; Acute Ischemic Stroke; Reperfusion; Emergency Treatment.

1. Introduction

Thrombo-occlusive cardiovascular disorders including heart attack and stroke comprise the first and third leading causes of mortality in the developed world [1, 2]. Chemical thrombolysis via clot disruptive medical agents are well accepted clinical-therapeutic options for treatment of acute arterial thrombotic obstructive events but are slow, expensive and not always successful [3 - 6].

It has been hypothesized by our group that non-invasive, Localized, Low Frequency Vibration (LLFV) massage in the low sonic ranges may offer a safe and practical adjunct to IV administered clot disruptive therapy to accelerate and ensure effective thrombolysis in the emergency treatment of heart attack, acute ischemic stroke, pulmonary emboli, and acute peripheral arterial thrombosis [7-9].

This hypothesis was derived in part by the work reported by Dubrul et al. and others in testing of a thrombolysis catheter (now the Trellis® 8 Peripheral Infusion System, Bacchus Medical - an FDA approved product for treatment of Deep Vein Thrombosis), that intraluminally applied LLFV (i.e. in direct contact within a fluid / clot medium) at frequencies of less than 100 Hz, greatly enhance clot dissolution with or without a thrombolytic drug agent [10, 11]. The potential of LLFV to disrupt clots is further supported by Wobser et al, who demonstrated the effectiveness of 50 to 500 Hz vibration in disruption of big blood coagula within the stomach via application of a vibrating gastro endoscopy probe, with the greater the intensity of the vibration the more complete the clot disruption [12]. Furthermore, Folts et al. has demonstrated that gentle tapping or shaking of acutely thrombosed coronary or carotid arteries (analogous to LLFV, but at a lower infrasonic frequency) reliably and immediately clears such thrombosis in open animal models [13].

To the Author's knowledge however, there has been no study to empirically demonstrate the hypothesis that LLFV when applied remote (or external) from a luminally situated blood clot (wherein the vibrations must first penetrate or transmit across an attenuation barrier to reach the intended target) would actually enhance clot dissolution. Hence a simple experiment was carried out whereby a low amplitude 50 Hz LLFV source was applied against the outer surface of a series of sealed 3 cc plastic syringes, each containing a blood clot immersed in Heparinized Saline solution, to assess whether the clots within the syringes would lyse to a greater degree than clots not treated by LLFV.

2. Methods

Work developing a suitable in-vitro vibrating system and subsequent experiment was performed in a private contracted lab setting (In-Vitro Laboratories, Burnaby, B.C., Canada). The test subjects (the Authors) who donated blood gave consent to the study, which had been approved by Institutional Review [14] in accordance to the U.S. Department of Health & Human Services Basic Policy for Protection of Human Research Subjects.

2.1 Equipment

A small hand-held vibrator (Hitachi Wand, HV – 250R), a low amplitude device advertised for use in gentle applications including facial and scalp massage, was connected to a power controller calibrated to enable LLFV emissions at 50 cycles per second. The amplitude emitted by the Hitachi Wand's oscillating contact node was visually estimated at about ~0.5 mm, as judged by the width of the node's vibrating corona during operation (which was barely discernible to the naked eye). This provided for a relatively gentle vibration within an order of magnitude to what might be experienced within a coronary, pulmonary, cerebral or peripheral artery if a substantially larger intensity of LLFV massage were to be applied non-invasively generally overlying the culprit vessel.

An LLFV frequency of 50 Hz was deemed appropriate for this experiment as it has been shown by Koiwa and his associates that non-invasive chest wall delivered diastolic timed vibration in the 50 Hz range improves cardiac performance and increases coronary flow in human volunteers with or without coronary artery

disease [15, 16]. Furthermore, 50 Hz is also within the range of frequencies which have found proven commercial use in vibrating catheter systems for disruption of blood clots in conjunction with thrombolytic drug agents [11]. Moreover, vibration at 50 Hz has been shown to fall within the resonance frequency spectrum of the heart's epi- myocardium [17- 19], hence once applied (such as to the chest wall), should theoretically provide a strong agitative effect to the heart muscle and coronary arteries thereupon regardless of overlying vibro-attenuating structures. Finally, LLFV generally in the low sonic ranges – including at 50 Hz - is known to produce convection currents in static fluid mediums [20, 21] which would be a desirable trait for improving mixing and effecting erosion or dissolution of solids (such as blood clots) in fluid mediums.

It should be emphasized that this paper does not deal with therapeutic ultrasound (a field widely studied for clot disruption [22 –25] but low frequency vibration at a much lower frequency and higher stroke amplitude.

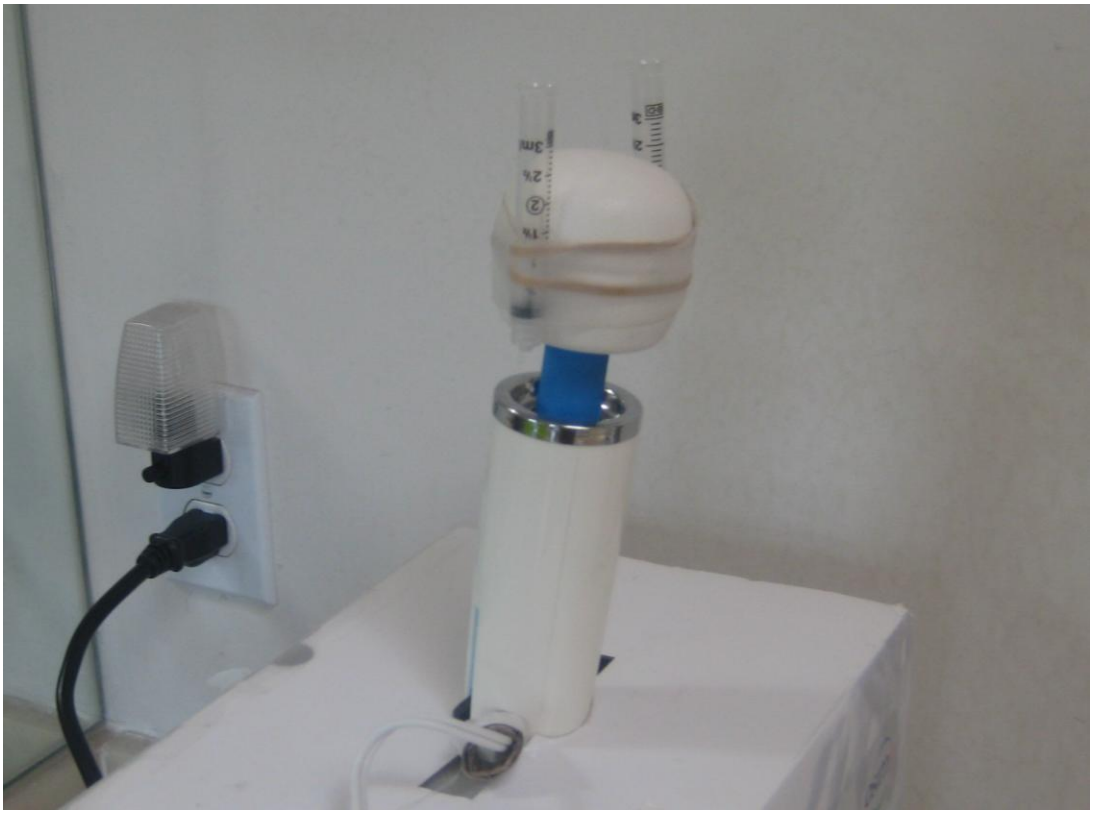

Figure 1. Our in-vitro model used to test the thrombo eroding effect of externally delivered LLFV. A low frequency Hitachi Wand massager in conjunction with a power controller (not seen) was used to vibrate blood clots immersed in Heparinized Saline solution contained within two 3 CC syringes per test run.

2.2 Harvesting and Treatment of Clots

The experiment was performed over two testing days via the assistance of two separate blood donors (i.e. the Authors, neither having acute illness or evidence of blood coagulation disorders). For each sample run a single fresh clot was produced by drawing an aliquot of donor blood to fill a 6 ml red top blood vacucontainer collection tube, which was immediately thereafter placed for 1 hr within the armpit of an investigator to keep the forming clot near core temperature. This produced a long, columnar retracted clot and free serum. Following the 1 hr incubation period, the serum within the vacucontainer was decanted

(a mesh sieve was used to assist in this process), and the clot was dispelled upon a dry plastic sheet. The clot was then gently rolled for a about 15 cm, allowed to air dry for one minute, and rolled an additional 15 cm to remove gross amounts of adherent serum. At that point the clot was cut lengthwise into four measured ~0.5 cm pieces, with the remainder of the clot discarded. The 0.5 cm pieces of clot were then each further roll dried for an additional 15 cm to remove additional adherent serum, which left four clots assembled left to right upon the plastic sheet. The two leftward versus rightward clots were then randomized whether they would receive LLFV treatment versus no LLFV. The clots were then each separately placed into a weighing boat and weighted on a digital analytic balance. After weighting, each clot was placed into the open end of a pre-labeled vertically held 3 ml syringe which had their expulsion end sealed through intraluminal placement of the rubber top of the syringe's plunger. The clots were allowed to freely travel down the syringe's lumen without further prodding or intervention, to whatever natural resting position within the syringe which gravity would dictate.

The syringes earmarked for LLFV were pre secured against the active reciprocating end of our vibrator (by use of tape and a double winded elastic band), with the syringes held upright and perpendicular against the oscillating end of the vibrator's contact node (see Figure 1 for a depiction of the vibration set up).

The control syringes were also held upright, but placed in a stationary container held free from any vibration or movement of any kind. Following loading the syringes with clot, 1.5 mls of 2 units/ml Heparinized Saline solution was slowly delivered to each syringe by way of a gentle injection against the inner surface of the syringes lumen to avoid direct mechanical perturbation of the clot. After this the open top ends of the syringes were securely covered with tape to prevent potential splatter. Mechanical thrombolysis was achieved by / vibro-percussing the syringes at 50 oscillations per second, produced by the attached vibrating node of the Hitachi Wand massager.

Figure 2. A comparison of a vibrated clot (left) versus a control clot (right). Both clots were roughly the same size prior to treatment.

To view the application of vibration to the blood clots, a video is available and can be accessed on the internet by the following link http://www.facebook.com/video/video.php?v=477245541631&saved (date last viewed August 21st, 2012).

After 20 minutes of treatment, the syringes were freed from their respective securements and decanted of fluid (again with help of a sieve mesh). The decantation phase for each syringe occurred in the same order by which the Heparinized Saline solution was first applied, in

an attempt to keep the total time of fluid clot exposure relatively constant between the syringes. Following decantation, the post treatment clots were then dispelled from their respective syringes upon a fresh dry plastic sheet, and then once again (in same manner as previous) rolled for 15 cm to remove excess adherent serum (see Figure 2).

Once "dried" the clots were each carefully placed upon a fresh weighing boat upon our digital scale for final weighting by a second investigator blinded to the type of treatment each clot had received. Percent % lysis of thrombus was derived as (pre clot weight – post clot weight) / pre clot weight X 100.

2.3 Statistics

A % clot lysis of greater than or equal to 20% was deemed successful in this experiment. The Fisher's exact test (2 X 2 contingency table – 2 tailed) was used for comparison of effective clot lysis between the vibration group versus the controls. A value of *P* less than or equal to .05 was considered statistically significant.

3. Results

3.1 Experimental results

Results are shown in tabular form (See TABLE 1).

Syringes exposed to LLFV in all cases showed a significant degree of bubbling and splatter (presumably depicting convection currents and turbulence within the system – see Video link embedded within this manuscript) – and the clear Heparinized Saline solution above and surrounding the clot quickly became uniformly red which signified that a process of clot dissolution (or erosion) was occurring.

Syringes which did not receive LLFV (i.e. controls) showed no movement within the fluid medium at all, and in all cases the clear Heparinized Saline solution remained substantially (virtually completely) clear even following 20 minutes of exposure of indwelling clot.

All resultant blood clots following LLFV treatment appeared relatively smoothly eroded, with no large chunks or visible fragmentation (or division) of the clots observed.

TABLE 1: Results of % clot lysis following 20 minute exposures of blood clots derived from two test donors treated with LLFV versus control.

Group	Subject #	#Samples	% Lysis	Avg. %Lysis
Vibration	1	6	34, 15, 21, 41, 26, 23	27.0%
Vibration	2	6	22, 30, 14, 29, 13, 30	23.0%
Control	1	6	6, 1, 2, 5, 7, 6	4.5%
Control	2	6	8, 6, 5, 1, 8, 5	5.5%
Vibration - totals	1 and 2	12		**25.0%**
Control - totals	1 and 2	12		**5.0%**

3.2 Statistical analysis

LLFV demonstrated statistically superior successful clot lysis in comparison to the controls which did not receive LLFV ($p < 0.0003$).

LLFV in conjunction with Heparinized Saline, on average demonstrated 5 X the amount of clot lysis as compared to control samples left stagnant, without vibration.

4. Discussion

Vibration at various frequencies has shown use in biological systems for disrupting tissue [26, 27]. This in-vitro experiment confirms that a low amplitude, *external, or extraluminal* application of low sonic LLFV (50 Hz, ~0.5 mm) applied to blood clots immersed in Heparized Saline solution in a plastic syringe model provides statistically superior clot dissolution (by successful % clot lysis) in comparison to clots which were not treated with LLFV. The results of this experiment were consistent with the results found by Dubrul et al in testing of an invasive vibrating catheter system [10], who showed that LLFV at frequencies of less than 100 Hz applied *intr*aluminally (i.e. directly proximate and / or within a clot / fluid interface – more resembling an invasive, intravascular therapy) generally doubled clot lysis in comparison to solutions not vibrated.

One explanation as to why LLFV may have displayed a superior lysing effect on blood clots in our experiment may be deduced through an examination of fluid mechanics. Firstly, it is known that LLFV causes convection currents in otherwise static fluid mediums [20, 21], which would offer disruptive shear forces and facilitate diffusion and advection of solutes (or solid / semi-solid fragments) interfacing with or existing within such a fluid. Secondly, it has been solidly established that diffusability or mix ability of a solute within a turbulent fluid is several orders of magnitude greater than what is seen in mere laminar (or absent) flow, due to the random velocity gradients within the fluid which cause eddies and vortices which greatly accelerate diffusion [28, 29]. The Author's thereby theorize that LLFV through the introduction of chaotic and random velocity shifts and shear patterns within the solution (which were evidenced in our experiment by random churning, bubbles and splattering within the vibrated syringes), may thereby have accelerated erosion of clot within the solution, and mixing of the Heparinized Saline solution within and upon the surface of the clot.

From a potential clinical perspective the Author's conjecture that the introduction of turbulent fluid – or blood - motion by LLFV (such as delivered in a non-invasive application) may theoretically in addition to effecting improved clot erosion offer additional benefits in improved mixing of systemically introduced drug agents (such a thrombolytics) down otherwise zero flow, stagnant thrombosed arterial systems. LLFV induced convection currents and vortices instilled at the boundary layer of a blocked artery (i.e. the boundary where the blocked, zero flow vessel meats the systemic, flowing circulation) would theoretically with a high degree of efficiency accelerate advection and diffusion of drug particles from the systemic circulation into a blocked vessel – thereby accelerating transport of the drugs to the culprit site of thrombosis whereby the drugs can take action.

That LLFV enhances mixing between two adjacent fluids is supported in fluid mechanics. It has been solidly established that mix ability of solutes and parallel fluids within an agitated fluid system is several orders of magnitude greater than what is seen in laminar (and especially absent) flow, due to the introduced random velocity and density gradients which cause eddies and vortices which greatly accelerate diffusion and mass transport [29, 30]. Indeed the correlation of increased mass transfer co-efficient between two fluids given an added LLFV application has been experimentally verified by Hancil et al [31]. Further, Oberti et al., have shown enhanced mixing of particulate laden fluids in articulating channels (analogous to articulating blood vessels) secondary to external LLFV [32, 33]. Accordingly, LLFV mixing devices such as produced by Resodyn ™ Acoustic Mixers (operable at a nominal frequency of 60 Hz) have found common use in industrial mixing of both fluids and solids.

It is also worth mentioning that cyclic stress and strain exerted on an endothelial lining of an artery (which would likely be induced through a prophesized LLFV application)[1], is predictive to cause liberation of beneficial mediators such as Nitric Oxide (NO) which is a potent vasodilator

[1] As LLFV is characterized by rapidly changing compressive and expansive mechanical forces in tissue, it is reasonable to postulate that LLFV would most likely expose the fluid and endothelial cells within the vasculature to such mechanical stimuli (i.e. cyclic stress and strain). Indeed, hydrodynamic analysis indicates that shear stress at the wall of vessels (including the coronaries) are significantly increased during bodily exposure to low frequency vibration in the 40 to 50 Hz range [34], hence the triggering of NO release by LLFV can therefore be hypothesized.

[35]. Indeed low frequency vibration stimuli has been shown to trigger NO release in various tissues [36 - 38], and can also induce a vasodilatory effect on arteries, particularly those in a state of heightened vascular tone or spasm [39- 41] (which is often the case in heart attack cases [42, 43] Additionally, researchers at Mt. Sinai Medical Centre (Miami, Florida) have recently studied the effects of low sonic vibration applied to the chest wall of rats which showed an up-regulation of endothelial derived NO Synthase (eNOS) with enhanced NO release, which has been discussed as a potential cardio protective mechanism in limitation of ischemic reperfusion injury [44, 45].

There were several limitations to this present study. First LLFV was not applied across a physiologic medium typical for overlying a site of arterial thrombosis (for example chest wall, neck, cranial bone, leg or arm tissue) but rather via external application adjacent the thin wall of a syringe. In mitigation of this factor, only a very small intensity (~0.5 mm oscillation) of LLFV was applied, which would be within an order of magnitude of intensity expected to typically reach a clot in-situ if LLFV at a substantially stronger intensity level where to be applied upon a non-invasive body surface such as a chest wall overlying a thrombosis site [16]. We are working on a future experiment whereby we will place a stronger vibrator against a chest wall sized attenuation barrier overlying a clot filled tube system to assess LLFV's potential to penetrate across such barrier and produce similar thrombo-clearing effects. Second we housed our blood clots within a syringe rather than an actual live artery. We felt however that as an early experiment to assess transluminal LLFV's basic ability to provide a clot disruptive effect this was adequate to provide a good "go–no go" hypothesis, in that if LLFV produced a negligible effect the application would most certainly also fail under clinical circumstances. Further, we unfortunately did not have authorization to use live animal or human systems (apart from human blood products) within the confines of our lab space so use of a physiologic artery was not an option. Third there was inherent experimental error associated with this experiment in that the degree of removal of adherent serum upon clots (post treatment) by rolling them on a plastic sheet and on and off the digital weight scale could not be made identical between test runs. However, great care was taken to manipulate and "dry" the treated clots in near identical fashion (i.e. the same degree of rolling), and the individual weighting the treated clots was blinded to the form of treatment the clots had received. Finally, the solution within the syringes was held at atmospheric pressure rather than a higher pressure more resembling that of an artery. The turbulent motion of the fluid in a pressurized system (and subsequent clot erosion) may therefore differ in a true arterial system – and the addition of arterial like fluid pressure will also be part of the next step we will take in evaluation of this technique.

The potential practical applications related to LLFV in the emergency non-invasive treatment of acute thrombotic arterial disorders are far reaching. Firstly, the Authors prophesize that in view of the prior clinical work of Koiwa et al. (whereby chest wall applied, 50 Hz, diastolic timed LLFV has been shown to improve cardiac performance and enhance coronary flow in humans [15]), and particularly in light of the results of our present study (that a gentle application of transluminal LLFV at 50 Hz facilitates clot dissolution), that chest wall diastolic timed 50 Hz LLFV massage may find use in the emergency treatment of ST Elevation Myocardial Infarction (with or without thrombolytic drug agents), whereby acute coronary thrombosis is present [42]. Similarly, a more gentle LLFV massage application (which wouldn't require diastolic timing) could for example be applied externally to the cranium and/ or neck in patient's as an adjunct to IV administered clot dissolving drug therapy in treatment of acute ischemic stroke. Furthermore, LLFV massage could also foresee ably be utilized in the non-invasive treatment of acute pulmonary emboli (particularly saddle emboli – a life threatening condition) or acute peripheral arterial thrombosis, as an alternative or bridge to surgery or catheter based removal techniques. Clinical trials would be required on all these fronts, in proving this technology's ultimate safety and effectiveness.

5. Conclusions

This study confirms that low amplitude LLFV at 50 Hz applied external to a clotted lumen enhances clot dissolution in Heparinized Saline Solution. It is postulated given the results of this study that LLFV may hold potential as a safe and practical adjunct to clot disruptive drug therapy in first line clearance of acute arterial thrombosis in the emergency setting.

It should be emphasized however that to the Author's knowledge there have been no clinical trials of using LLFV in treatment of acute arterial thrombosis, hence the safety and efficacy of this technique remains unclear (particularly in conjunction with thrombolytic or other potent clot disruptive agents), and cannot at this time be warranted. Also, it should be mentioned that use of LLFV is not recommended in treatment of Deep Vein Thrombosis, as sudden mobilization of clot in this scenario could lead to a worsening condition (such as pulmonary embolus or stroke).

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Disclosure

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