Evidence for the Molecular, Cellular and Physiological Effects of Microstreaming and Cavitation at MHz and kHz Ultrasound Frequencies

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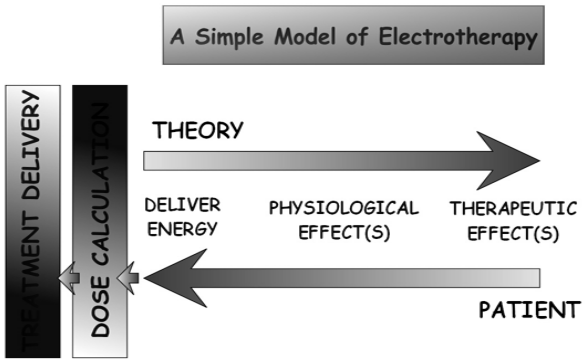
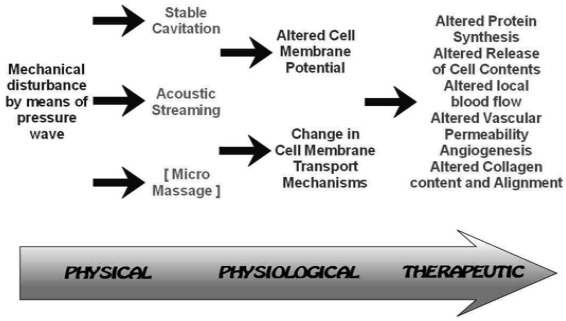
Acoustic Streaming and Cavitation in the Context of Non-Thermal Ultrasound

- The bioeffects of Ultrasound, used in a 'non-thermal' context are largely explained on the basis of both **CAVITATION** and **MICROSTREAMING**
- The **APPLIED ENERGY** brings about **PHYSICAL EFFECTS** which in turn generate **PHYSIOLOGICAL CHANGES** which we use as a means to a **THERAPEUTIC EFFECT**



Ultrasound Mechanisms



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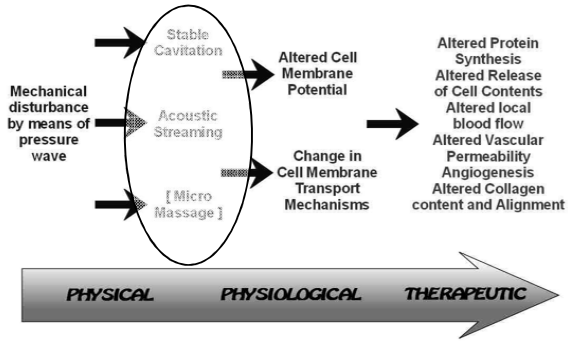


Cavitation and Acoustic Streaming

- Although these phenomena exist on an independent basis, they are strongly linked and are effectively inter-related to a greater extent
- There is some confusion with regards terminology, and the physicists, engineers and therapists tend to adopt different versions of this language (as ever!)

Evidence . . . . . .

- There is a **LOT** of evidence out there with regards these phenomena **BUT** the majority does **NOT** come from the therapy literature and **MANY** aspects of this work are related to **scanning ultrasound, HIFU (high intensity focused ultrasound), sonophoresis, sonoporation and gene transfection**
- Significant proportion of the research is done in vitro which also raises issues of '**transfer**' to the clinical environment

HIFU (High Intensity Focused Ultrasound)

- HIFU (sometimes FUS or HIFUS) is a highly precise medical procedure using **high-intensity focused ultrasound to heat and destroy pathogenic tissue rapidly**.
- As an acoustic wave propagates through the tissue, part of it is absorbed and converted to heat. With focused beams, a very small focus can be achieved deep in tissues. When hot enough, the tissue is thermally coagulated.
- At high enough acoustic intensities, cavitation will occur.
- Microbubbles produced in the field oscillate and grow, and eventually implode (inertial or transient cavitation).
- During inertial cavitation, very high temperatures inside the bubbles occur, and the collapse is associated with a shock wave and jets that can mechanically damage tissue.

Sonophoresis

- ***Sonophoresis*** is a process that exponentially increases the absorption of topical compounds (transdermal delivery) into the epidermis, dermis and skin appendages.
- ***Sonophoresis*** occurs when ultrasound stimulates 'micro-vibrations' within the skin epidermis and increase the overall kinetic energy of molecules making up topical agents.
- Used to deliver drugs through the skin.
- The drugs are mixed with a coupling agent (gel, cream, ointment).

Sonoporation

- **Sonoporation** utilizes the interaction of ultrasound (US) with contrast agents (UCAs) to temporarily permeabilize the cell membrane allowing for the uptake of DNA, drugs, and other therapeutic compounds from the extracellular environment.
- This membrane alteration is **transient**, leaving the compound trapped inside the cell after US exposure.
- **Sonoporation** is a developing drug delivery and gene therapy technique

Forbes, 2008

Gene Transfection

- **Transfection** is the delivery of DNA, RNA, proteins, and macromolecules into cells.
- Goals for transfection include the study of gene regulation as well as protein expression and function.

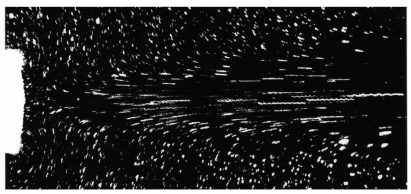
Acoustic Streaming

Acoustic Streaming

- Acoustic Streaming is the **movement of fluid** due to an ultrasound wave.
- The movement occurs in the direction of the beam, away from the transducer and is due to the energy transfer from the US wave to the fluid
- Is the opposite of sound generation by a flow.

Clarke et al 2004, Barnett 2001, Shi et al 2002, Zauher et al 1998, Duck 2008

Acoustic Streaming in Water (Duck 2008)



This is in effect a 'bulk streaming' – movement of fluid in a single direction - whereas in therapy context, the important element is almost certainly microstreaming which occurs adjacent to an oscillating source/surface and is therefore most strongly associated with cavitation.

The controversy is that if cavitation does not occur in vivo, then microstreaming will not happen either – only the less powerful bulk streaming

Acoustic Streaming

- It has importance for several established and developing techniques in diagnostic US, but is also postulated to have a role in US as therapy
- At low US intensities, acoustic streaming is likely to be significant, but at higher levels, heating and acoustic cavitation will predominate (ter Haar, 2007)
- Harle and Mayia (2004) for example considered whether it was a cavitation or a streaming effect that altered TGF-b expression following low power US. It was determined that STREAMING was the more important component.

Acoustic Streaming

- Baker et al (2001) suggest that
- '. . . . The frequently described biophysical effects of ultrasound either do not occur *in vivo* under therapeutic conditions or have not been proven to have a clinical effect under these conditions . . . . . '
- There remains considerable controversy with regards the 'transfer' of in vitro research data directly to the clinical environment and although there has been new evidence since 2001 it may not have that much impact on the transfer issue

Microstreaming Concept

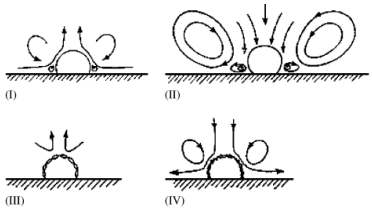


Fig. 3. Microstreaming pattern near a pulsating hemisphere bubble trapped at a plate wall (Elder, 1959).

cited in Wu (2007)
Progress in Biophysics and Molecular Biology 93; 363-373

Acoustic Microstreaming

VanBavel 2007
Progress in Biophysics and Molecular Biology 93; 374-383

- Describes the difference between gross acoustic streaming and microstreaming
- The microstreaming phenomenon occurs near microbubbles (2-5 micron diameter) – such as US contrast agents
- No evidence is identified that microstreaming can occur without the microbubbles being present

Plasma Membrane Permeability

Schlicher et al (2006)
Ultrasound in Medicine & Biology 32(6);915-924

- In vitro experiment that clearly demonstrates the change in membrane permeability as a result of a microstreaming / cavitation (microbubble) effect
- Different membrane phenomena evaluated, but the most interesting aspect of this work was the demonstration of (temporary) membrane wounds which allowed the permeability change

Membrane permeability (contd)

- The results of their work show that the cavitation generated by US facilitated the incorporation of macromolecules through repairable micron-scale disruptions in the cell membrane
- These disruptions have a life span of apx 1 minute following US exposure and then repair
- **BUT** dependent on a cavitation effect to achieve this change

ter Haar (2007)

- Identifies the same phenomenon :
- Microstreaming is set up in the fluids around acoustically driven bubbles.
- This leads to shear stresses on cell membranes in the vicinity, which may create transient pores through which ions and molecules may be transported
- **BUT** it is still dependent on a cavitation effect

Microstreaming without cavitation?

- Unable to identify **any** evidence in the physics or biophysics literature which establishes a microstreaming effect, in vivo, **without** a cavitation event
- It is widely postulated, assumed and considered entirely possible – maybe at a smaller level (??nano) – but not established

Cavitation

Cavitation

- Lots of definitions, many are complex and unwieldy
- Baker et al (2001) suggest that in the context of therapy, cavitation can be defined as
- ' . . . the formation of tiny gas bubbles in the tissues as a result of ultrasound vibration . . .'

Bioeffects of Cavitation Miller et al (1997)

- Defines cavitation as ' . . the interaction between an ultrasonic field in a liquid and a gaseous inclusion . . . '
- There are 2 (related) categories :
- **GAS BODY ACTIVATION** (was previously known as STABLE CAVITATION)
- **INERTIAL CAVITATION** (was previously known as TRANSIENT or UNSTABLE CAVITATION)

Gas Body Activation (Stable Cavitation)

- Only demands a relatively low US intensity to activate a pre-existing gas body
- The gas body undergoes periodic and regular changes in volume in response to the applied acoustic pressure

Miller et al 1987, 1997

- BUT without pre-existing gas bodies in the tissues, is it a realistic effect of therapy US?

Inertial Cavitation (Transient/Unstable)

- A bubble undergoes periodic changes in volume in concert with the applied acoustic pressures
- Rapidly increases in size, becomes unstable and then implodes violently
- Free radical generation occurs, as does light production (sonoluminescence) and hydrolysis together with structural cell damage

Miller et al 1987, 1997

In Vitro – In Vivo

- Most of the high quality work is done with cell suspensions ***in vitro***
- Rarely done with multicellular aggregates or whole tissues
- Almost never done ***in vivo***
- Problem of results transfer from one situation to the other – need to be careful!

In Vitro systems (after Miller et al 1996)

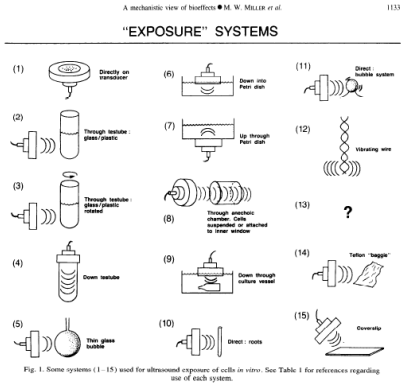


Fig. 1. Some systems (1–15) used for ultrasound exposure of cells *in vitro*. See Table 1 for references regarding use of each system.

Cavitation – the clinical reality

- ' . . . the medical application of pulsed ultrasound for lithotripsy and diagnostic imaging with contrast agents are the most likely to involve bioeffects induced by gas body activation or inertial cavitation . . . . '

Miller (2007)
Progress in Biophysics and Molecular Biology (93) 314-330

- The US either has to be of high intensity (as with the lithotropsy) or have US contrast agents (microbubbles) in place at lower intensity

Clinical reality of cavitation : Animal (in vivo) Study

Ogurtan et al (2002)
The Veterinary Journal 164; 280-287

- Research (in vivo) looking at the effect of US on growth plates in young rabbits
- 0.2 and 0.5 W cm^{-2} exposures for varying time periods (1MHz, Pulsed 1:4, 5 minutes daily)
- Apart from looking for growth plate behaviour changes, they also looked for cavitation effects in the cells (flourescent microscopy) but identified **NO CAVITATION EFFECTS** on the examined samples
- Propose the any changes that they observed were related to the mechanical disturbance of the cells rather than being attributable to a cavitation effect.

Claims versus the Evidence

- Almost all standard texts and many research papers attribute the effects of US in a non-thermal mode to be attributable to both stable cavitation and microstreaming (using various different terminologies)
- Whilst there is evidence that both effects DO occur as a result of the application of US energy, there is a leap of faith involved with the transition from the ***in vitro*** to the ***in vivo*** arenas

LIPUS – another fly in the ointment . . . . . .

- The evidence for the clinical efficacy of **LIPUS** (low intensity pulsed ultrasound) is **VERY** strong and growing month by month in terms of published research
- **BUT** it uses **EVEN LOWER** doses than classic 'non-thermal' US
- Factor of at least 3 x lower intensity
- If non thermal US not working through acoustic streaming and cavitation, how can LIPUS be utilising this mechanism?

Possibilities . . . . . .

- It is possible that both effect DO occur in the ***in vivo*** world, but have not yet been adequately measured (an experimental nightmare)
- It is possible that although gross cavitation does not occur, there might be a microcavitation (or even a nano cavitation) effect which comes into play
- It is possible that neither occur in clinical US but that does not negate the biological effects of the therapy

Conclusion

- **There is evidence that cavitation and microstreaming are associated with ultrasound energy application**
- **The microstreaming appears to be dependent on the cavitation**
- **There is in vitro evidence for these effects**
- **There is almost nothing by way of in vivo evidence**
- **Widely assumed to exist – though may be an erroneous assumption**

Thank You

pdf file of the slides and all references available at :
www.electrotherapy.org/ISEPA.html