Mini-Symposia Title:

Low Intensity Focused Ultrasound: engineering developments and therapeutic applications.

Mini-Symposia Organizer Name & Affiliation:

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Mini-Symposia Speaker Name & Affiliation 5:

Elisa E. Konofagou, Columbia University, USA

Mini-Symposia Speaker Name & Affiliation 6:

Leonardo Ricotti, Scuola Superiore Sant'Anna, IT

Theme:

- ○ 01. Biomedical Signal Processing
- ○ 02. Biomedical Imaging and Image Processing
- ○ 03. Micro/ Nano-bioengineering; Cellular/ Tissue Engineering &
- ○ 04. Computational Systems & Synthetic Biology; Multiscale modeling
- ○ 05. Cardiovascular and Respiratory Systems Engineering
- ○ 06. Neural and Rehabilitation Engineering
- ○ 07. Biomedical Sensors and Wearable Systems
- ○ 08. Biorobotics and Biomechanics
- ◉ 09. Therapeutic & Diagnostic Systems and Technologies
- ○ 10. Biomedical & Health Informatics
- ○ 11. Biomedical Engineering Education and Society
- ○ 12. Translational Engineering for Healthcare Innovation and Commercialization

Mini-Symposia Synopsis— Max 2000 Characters

Very recent technological advances have led to the development of human-compatible Low Intensity Focused Ultrasound (LIFU) technologies, which are increasingly gaining ground as an advantageous alternative to conventional treatment of a variety of neurological and oncological conditions. Accordingly, several clinical trials using LIFU are currently underway. As an example, LIFU can effect both as excitatory and inhibitory neuromodulation, and is currently being explored in the treatment of consciousness disorders in acute brain injury as well as for deep brain stimulation. Other exciting applications include the use of microbubbles, which can be combined with LIFU, to temporarily modulate vascular permeability and hence greatly facilitate the targeted release compounds for the delivery of substances which would otherwise not be able to cross the blood-brain barrier (BBB).

This mini symposium will cover both the engineering principles and safety aspects, as well as challenges and therapeutic potential of LIFU in human applications. We will first focus on experimental and modeling research demonstrating transient BBB opening through FUS. We will then present the latest developments in MRI-guided FUS technologies for trasnferring macromolecules and genetic material to target structures or even specific cell types (e.g. "sonoselective" transfection of cerebrovascular endothelium). Successively, we will explore the mechanisms underlying FUS-induced neuromodulation both in humans and primates, like e.g. using FUS to enhance cognitive performance in healthy subjects as well as in Mild Cognitive Impartment as well as to regulate emotions. Additional avenues for the biomedical exploitation of low intensity FUS (e.g. in regenerative medicine) will also constitute an integral part of the symposium.

To the Blood Brain-Barrier and Beyond: MRI-Guided Gene Delivery with Low Intensity Focused Ultrasound

Richard J. Price, University of Virginia

Abstract— **Our objective is to engineer MR image-guided focused ultrasound (FUS) approaches for gene delivery to the brain. Emphasis is placed on combining low-intensity focused ultrasound pulsing sequences and gene delivery formulations to most effectively transfer genetic material to target structures or even specific cell types.**

I. INTRODUCTION

The blood-brain barrier (BBB) limits gene therapy treatments of central nervous system (CNS) disorders. Focused ultrasound (FUS) activation of contrast agent microbubbles may be used to overcome this barrier, as this approach non-invasively and temporarily disrupts the BBB. Further, we postulate that tuning focused ultrasound parameters can also facilitate the uptake of genes by only the cerebrovascular endothelium.

II. METHODS

MR image-guided focused ultrasound drives the oscillation of intravenously administered microbubbles (MBs) flowing through cerebral microcirculation. This transiently opens the blood-brain (BBB) and/or blood-tumor (BTB) barriers to permit drug and gene bearing nanoparticle (NP) delivery. Many of our approaches utilize systemically-administered non-viral nanoparticles with penetrating properties for gene delivery deep into tissue. Here, we also attempt "sonoselective" transfection of cerebrovascular endothelium, wherein transgene delivery is localized to the vessel wall and achieved using very low focused ultrasound pressure (i.e. sub-BBB opening) with plasmid-coated MBs.

III. RESULTS

We have previously used MR image-guided FUS and MBs in combination with NPs to transfect neural tissue (1), reduce glioma invasiveness (2), and treat PD rats (3). Here, we show that delivery of nanoparticles to primary and metastatic brain tumors elicits targeted tumor transfection (Figure 1A), with a significant component attributed to augmented interstitial flow. Further, we show that cerebrovascular endothelium may be selectively transfected without detectable BBB opening (Fig 1B), which may be useful for conditions wherein even transient BBB disruption could be contraindicated.

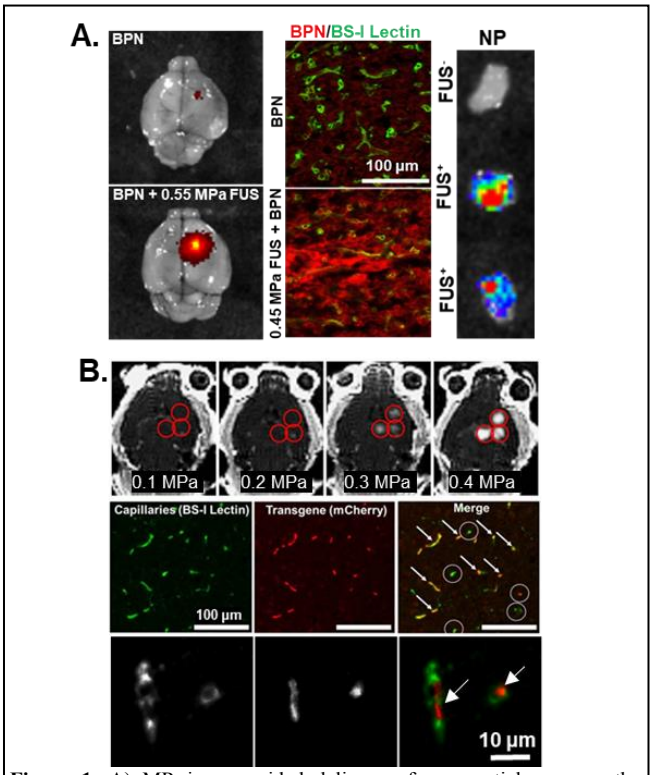

Figure 1. A) MR image-guided delivery of nanoparticles across the blood-tumor barrier (left, middle), leading to tumor transfection (right). B) At very low FUS pressures (i.e. 0.1 MPa), the BBB remains closed (top) and "sonoselective" transfection of cerebrovascular endothelium is achieved (middle and bottom; arrows denote transgene co-localization with endothelial cells.

IV. DISCUSSION & CONCLUSION

MR image-guided FUS is a disruptive platform technology for drug and gene delivery to the brain. As clinical BBB/BTB opening trials move forward, a continued emphasis on the parallel pre-clinical development of innovative new delivery strategies will be imperative.

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Focused Ultrasound-mediated drug delivery through the Blood-Brain Barrier: an empirical and theoretical Study

Allegra Conti, CEA/NeuroSpin, France, and University of Rome Tor Vergata, Italy

Abstract— **Low Intensity Focused Ultrasound (FUS)-induced Blood-Brain Barrier (BBB) permeabilization is a promising technique used to deliver molecules within brain tissue. So far none has modelized, on a macroscopic space and time scales, the effects of FUS on both BBB properties and on the distributions of the compounds getting in the brain. Here we introduce two models addressing these goals, validated by drug delivery experiments performed *in vivo*.**

I. INTRODUCTION

Despite increasing efforts and encouraging results, drug delivery to Central Nervous System (CNR) remains a challenging task. Indeed, the blood-brain barrier (BBB) not only prevents neurotoxic substances from entering the brain, but also limits the passage of therapeutic products to the CNS. Many studies have demonstrated that pulsed focused ultrasound (FUS) combined with circulating microbubbles can permeate the BBB in a reversible manner. Here we present two models able to depict the effects of BBB-permeabilization on both tissue properties and particles distribution. Specifically, the first model is able to describe the BBB closure time ($t_{1/2}$) as a function of both the diameter of delivered molecules (d_H) and the BBB gap sizes (σ_0) created by FUS application [1]. The second model is able to depict particles distribution within brain tissue, taking into account tissue and particle properties as well as acoustic parameters [2]. Both these models have been validated through BBB-opening experiments performed *in vivo* on rats.

II. METHODS

In 2012, our group demonstrated that the BBB remains permeable to small MRI contrast agents up to 24 h after ultrasound application and also that this duration was dependent on nanoparticle size, deriving a simple theoretical model explaining these observations [3]. However, in that paper the expression of the BBB closure time as a function of the size of d_H could not be related to the other physical parameters of the model. Here, we present a formal solution, finding the same expression of $t_{1/2}$ already published and linking $t_{1/2}$ to relevant physical variables such as the molecular hydrodynamic diameter d_H, the BBB closure rate k and the BBB gap sizes σ_0. This equation has been validated by fitting the data presented in [3], obtained after inducing in 47 rats, the permeabilization of the BBB through a Magnetic Resonance-guided FUS (MRgFUS) system (1minute sonication, 3% Duty Cycle (DC), central frequency 1.5 MHz, acoustic pressure (AP) 0.45 MPa). Within 24 hours after sonication, different MR Contrast Agents (CA) have been injected in order to evaluate the BBB closure time ($t_{1/2}$) as a function of their sizes. The second model depicts CA concentration ([CA]) getting in the brain after FUS-induced BBB disruption.

This model describes particles' Apparent Diffusion Coefficient (ADC), and takes into account the AP used to permeabilize the BBB, the gaps created between endothelial cells, the BBB closure time as well as the size of the delivered molecules [2]. Experiments have been performed in the same way as the previous ones. Our model has been fitted on [CA]-maps recorded through MRI. By estimating CA Free Diffusion Coefficients from in-vitro studies, and the ADCs from our model, we have assessed tortuosity (λ) in the right striatum of 9 rats.

III. RESULTS

In Figure (A) our first model (in red) is compared to the experimental data (black). Our model is able to estimate $t_{1/2}$ with an error smaller than 5% for all the delivered molecules. In Fig. (B) experimental and theoretical CA-distributions are compared. The second model we propose is able to depict molecular diffusion after BBB-opening within a long time after sonication (more than 1 hour). More quantitatively the λ values obtained by using the model, and comprised between 1.2 and 1.3 agrees with the literature. Indeed, the expected value of brain tortuosity after a FUS induced BBB permeabilization experiment is equal to 1.3 [4].

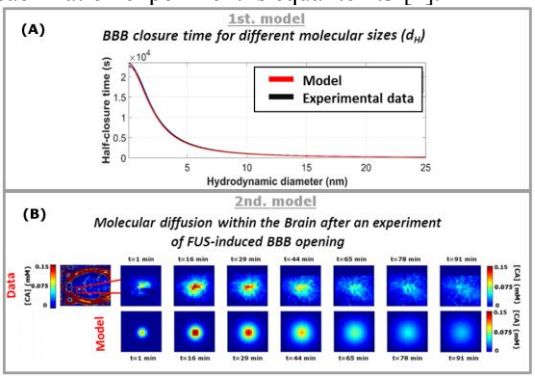

Figure 1. Comparisons between the models and the experimental data.

IV. DISCUSSION & CONCLUSION

The agreement between our models and experimental data, demonstrates that both models are suitable to depict different aspects of FUS-induced BBB opening experiments. Our first model suggests that the measurement of two ($t_{1/2}$, d_H) variables is sufficient to determine k and σ_0 for given experimental conditions of BBB opening and the second one is able to predict particles distributions obtained through an experiment of FUS-mediated drug delivery.

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Sustained and specific manipulations of oculomotor performance in primates by repetitive focused transcranial ultrasound stimulations.

Pierre Pouget, ICM, CNRS UMR 7225, Paris, FRANCE

***Abstract*— Using a repetitive transcranial focused ultrasound stimulation directed toward the oculomotor regions within the frontal cortex, we provide evidence for sustained, reversible and specific modulation of oculomotor behavior in non-human primates.**

I. INTRODUCTION

Transcranial Ultrasound Stimulation (TUS) allows safe, non-invasive stimulation of the brain and has emerged as a novel and valuable tool for studying brain function in humans and animals. The use of TUS gathered momentum in the first decade of the new millennium, using low frequency, low intensity ultrasound waves to stimulate rodent primary motor cortex to generate motor responseswithout damaging brain tissue [1-2]. In the same vein, TUS has been shown to immediately alter electromyographic and electroencephalographic measurements in sheep and humans, and suppress somatosensory evoked potentials in swines. In awake non-human primates, a study by our group has shown that single pulses of TUS could modulate visuomotor behavior and further electrophysiological recordings of neuronal discharge demonstrated brief modulating effects (~100msec) of TUS on single neurons. Whether such modulation due to TUS can be temporally extended and controlled remained to be demonstrated.

II. METHODS

In this study, animals performed a total of 40 sessions (10 stimulated sessions “rTUS sessions” and 10 non-stimulated sessions “no-rTUS sessions” for the region of interest Frontal Eye Field (FEF) and Supplementary Eye Field (SEF) and for control region. Each experimental session contained, after one non-recording warm-up block, a total of 7 blocks of 100 antisaccades (50 each side randomly distributed). The repetitive Transcranial Focused Ultrasound Stimulation (rTUS) was delivered when the first block of trials had been completed (defined as pre-rTUS block, the 6 following as post-rTUS).

A single element focused ultrasound transducer (H115, Sonic Concept, Bothell, WA, USA; central frequency 250KHz, diameter 64mm, FD# 1) was used in those experiments. A coupling cone (C103, Sonic Concepts, Bothell, WA, USA) filled with degassed water was placed between the transducer and the animal head. The transducer was fixed on a mechanical arm with four rotation axes (Viewmaster LCD, Osmond Ergonomics, Wimborne, UK) to enable flexible positioning of the transducer over the head. A thin layer of echographic gel (Aquasonic 100, Parker Laboratories Inc., Fairfield, NJ, USA) cone to ensure acoustic coupling (Figure 1B).

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The ultrasound frequency was set to 320 kHz. The pulse duration was 30 ms with a rise and fall time set to 1ms to avoid abrupt changes in pressure. The pulse repetition frequency was set to 10 Hz and the total sonication time was 20 s. The signal was generated by a TiePie generator (Handyscope HS5). A 75-watt amplifier (75A250A, Amplifier Research, Souderton, PA) was then used to deliver the required power to the transducer and the input voltage of the transducer was monitored using a voltage probe (P6139A, Tektronix, Melrose, MA) connected to a TiePie oscilloscope.

III. RESULTS

The analysis of the time courses per blocks (Figure 1) showed a significant decrease of mean latencies for both contralateral an ipsilateral antisaccades for FEF stimulations, mostly for the first block of trial post-rTUS but none variations with the visual cortex stimulations. Together all these results on monkey S performances suggested that the rTUS have more impact on normal antisaccades rather than on express antisaccades.

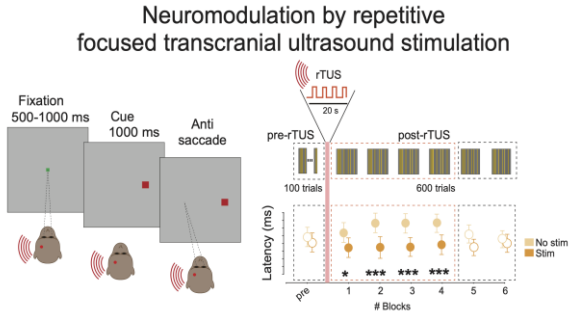

Figure 1. Animal performed antisaccade task with and without rTUS stimulation (left). Saccade latency were significantly reduced for up to 4 blocks (~20 minutes) after rTUS compared to no stimulation.

IV. DISCUSSION & CONCLUSION

Our study demonstrates the feasibility of using focused ultrasound to modulate visual behavior for a sustained period of several minutes (~20) in the awake non-human primate brain.

ACKNOWLEDGMENT

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Low Intensity Focused Ultrasound: A Possible Non-Invasive Cognitive Neural Prosthetic

Taylor Kuhn, PhD, University of California Los Angeles

Abstract— **Low Intensity Focused Ultrasound Pulsation appears to be able to selectively increase regional perfusion and functional connectivity of targeted brain regions. As such, it may have clinical utility for modulating neural function and affecting associated cognitive and emotional health.**

I. INTRODUCTION

Both noninvasive and invasive neuromodulation tools have been shown to alter brain function and increase neural activity. There is strong evidence that low intensity focused ultrasound pulsation (LIFUP) directly increases neural activity: early histology and animal studies of LIFUP have demonstrated its ability to produce reversible physiologic effects on neuron clusters, including increased activity in targeted areas[1]. Further, animal and human studies have used fMRI to confirm the effects of LIFUP on neural activity. In studies using real time fMRI of rabbits, LIFUP produced increased activation in the blood oxygenation level dependent (BOLD) signal in regional brain targets (e.g.V1)[2]. More recent fMRI studies in humans demonstrated the ability of LIFUP to focally increase BOLD activity in primary somatosensory[3], primary visual[4] and simultaneous primary and secondary somatosensory cortices[5]. Importantly, the study involving LIFUP stimulation of V1 reported LIFUP-evoked increased BOLD fMRI signal in both V1 and functionally associated networks. Importantly, more work on the safety and clinical utility of LIFUP remains before LIFUP can be established as a translational clinical tool.

II. METHODS

Our group performed a safety study where we targeted the medial temporal lobe with LIFUP in pre-surgical temporal lobe epilepsy patients. LIFUP, at insenties ranging from 720mW up to 5660mW, was administered two weeks prior to surgical resection of the temporal lobe that received LIFUP. Additionally, we are conducting two interrelated studies investigation the use of LIFUP as a potential cognitive neural prosthetic in healthy adult humans affecting: memory via exciting the entorhinal cortex (ERc); and anxiety via dysrupting the amygdala (AG). The study design is a randomized, double-blind within-subject cross-over.

III. RESULTS

During the safety study, no side effects were seen and post-lobectomy histology showed no negative effects on the excised tissue, including no evidence of heating or cavitation damage. Through the double-blind studies we found that LIFUP selectively increased blood perfusion in the targeted region, using Arterial Spin Labeling (ASL) MRI. In line with this finding, we also discovered that LIFUP focally increased BOLD activity in the targeted regions and functionally connected regions. For example, LIFUP selectively increased perfusion in the ERc and increased BOLD activity in the ERc and functionally connected regions including bilateral hippocampus, cingulate and anterior thalamic nucleus. Using the amygdala as a control target, we confirmed that these findings were selective to the brain region targeted using LIFUP. When targeting the amygdala, increased perfusion was seen in the amygdala and not the ERc. Similarly, LIFUP decreased amygdala connectivity (e.g. with prefrontal cortex) and not ERc connectivity, and vice versa. Said differently, thus far we are seeing a double dissociation between perfusion and FC results based on the region targeted with LIFUP which strongly suggests that the modulatory effects of LIFUP are focal and directly related to the targeted region.

We are now moving into a study using LIFUP sonication of the ERc in patients with amnestic mild cognitive impairment to determine if LIFUP can have similar effects on perfusion and connectivity as well as associated memory changes in a neurodegenerative group. Findings from this study will be presented during the mini-symposium.

IV. DISCUSSION & CONCLUSION

There is early confirmation that affecting local neural activity with ultrasound impacts functionally connected circuits. Overall, these preliminary results suggest that LIFUP: using our parameters appears safe in humans; targeting is very accurate; can be used to selectively increase regional blood flow in the targeted brain region; can selectively affect functional connectivity of the targeted brain region and its functional network in the desired direction (i.e. increased or decreased connectivity); may affect behavioral performance associated with the targeted region.

ACKNOWLEDGMENT

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Alzheimer's Association Zenith Award

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Clinical feasibility of focused ultrasound for peripheral nerve modulation

Hermes A. S. Kamimura and Elisa E. Konofagou, Columbia University

***Abstract*— Focused ultrasound (FUS) has been demonstrated capable of modulating peripheral nerve activity in animals. In this study, clinical feasibility of PNS modulation was assessed in healthy human subjects for both sensation stimulation and suppression. The suppression of electrically elicited somatosensory evoked potentials shows the potential analgesic effect of FUS, which may be used to alleviate refractory pain.**

V. INTRODUCTION

Focused ultrasound (FUS) can directly and noninvasively activate or inhibit peripheral nerve responses. Previous studies with mice have shown that the acoustic radiation force generated by single pulses of FUS can activate the nerve and produce downstream motor responses [1]. Conversely, inhibitory responses are observed through localized nerve heating using repeated pulses [2]. The capability of FUS in modulating nerve function may potentially offer a noninvasive (transdermal) alternative to block hyperactivity through the peripheral nerve to alleviate chronic pain. In this study, we developed a clinical setup and evaluated motor and sensory responses in healthy subjects associated with the FUS stimulation of the median nerve.

VI. METHODS

This study was conducted under protocol approved by the institutional review board of Columbia University. The clinical setup (Fig. 1) consisted of a 1.1-MHz FUS transducer and an imaging probe (P12-5, ATL/Philips, USA) both driven by a Verasonics Vantage system (256 channels, Vantage, Verasonics, USA). A robotic arm (JACO, Kinova, Canada) was used to place the transducer on the subject's forearm. Targeting of the median nerve was performed following anatomical evaluation using B-mode images and confirmed with displacement imaging in real-time. Single FUS pulses were delivered blindly followed by electromyography and electroencephalography recordings of finger motor responses and somatosensory evoked potentials (SSEPs), respectively. A subset of subjects underwent concurrent electrical stimulation following same evaluation. Subjective evaluation of sensory responses was reported by the volunteers in addition to the recorded quantitative measurements.

VII. RESULTS

The stimulation of the nerve trunk generated sensations (tingling, poke, pulse travelling) along the median nerve and innervated areas of the palm and fingers. In addition to that,

Figure 1. Clinical setup. (a) Table of values tested in healthy volunteers. (b) Clinical setup using a robotic arm to position the transducers. (c) Button for volunteer reporting of sensation. (d) Prototype 1 using water bath. (e) Prototype II using bladder only (no water bath). (f) Imaging guidance using B-mode imaging showing the median nerve.

subjects reported motor-related sensation such as light cramp, spasm, and twitches, but they were not detected in EMG or SSEP recordings. Interestingly, concurrent application of FUS and transcutaneous electrical stimulation both on the median nerve generated SSEP signals with lower amplitudes in comparison to signals obtained from electrical stimulation alone. Repeated sessions (5) of FUS and electrical stimulations generated a significant SSEP signal attenuation of about 20%.

VIII. DISCUSSION & CONCLUSION

FUS was demonstrated safe in repeated sessions and capable of modulating human peripheral nerve. The decrease in the sensory responses indicates a potential analgesic effect of FUS, which will be explored for pain relief.

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Low intensity pulsed ultrasound for regenerative medicine

Leonardo Ricotti, *Member, IEEE*

***Abstract*— This paper and the related talk aim at describing the potential of ultrasonic stimulation, alone or in combination with smart nanomaterials, to induce regenerative phenomena in cells and tissues.**

I. LIPUS STIMULATION

Ultrasound (US) is raising a great interest in the biomedical community also as a tool for treating pathologies and for regenerating tissues. In this domain, low-intensity pulsed ultrasound stimulation (LIPUS) is being used as an effective healing/regenerative tool for both hard and soft tissues and it has been proposed as a means to modulate inflammation and neural functions [1].

LIPUS is clinically appealing because it is a safe and non-invasive stimulation paradigm, able to transfer mechanical energy through the body in a wireless way without incisions. It can be used to trigger different phenomena, both mechanical and thermal ones, in a versatile way. However, while the benefits of LIPUS have been repeatedly demonstrated *in vitro* and *in vivo*, studies are too often conducted without taking care of attenuations, reflections and other error sources in the stimulation system. Thus, a clear correlation between ultrasonic dose and beneficial bioeffects has been scarcely investigated, so far.

A research step that will enable an important advancement in this field actually concerns the quantification and the precise control of LIPUS-related parameters, and their correlation with cell and tissue outcomes, as well as intracellular mechanisms. We explored the engineering of LIPUS technology, first by analyzing the pressure field generated by transducers and taking into account the differences in terms of intensity and field shape found at different frequencies. Second, we developed a*d hoc* tools to guarantee a precisely controlled transmission of such pressure waves to cells, without attenuations and reflections (Figure 1). This allowed us to identify "optimal" stimulation parameters promoting the proliferation and differentiation of myoblasts, thus finely tuning this tool to maximize muscle regeneration [2]. The search for optimal stimulation parameters is ongoing on other cell/tissue types, *e.g.* macrophages and lymphocytes to tune inflammatory processes and on stem cells, to verify the possibility to direct their fate.

II. SYNERGY BETWEEN LIPUS AND SMART NANOMATERIALS

Piezoelectric nanomaterials allow to convert a mechanical deformation (such as the one induced by a pressure wave) into an electrical signal and *viceversa*.

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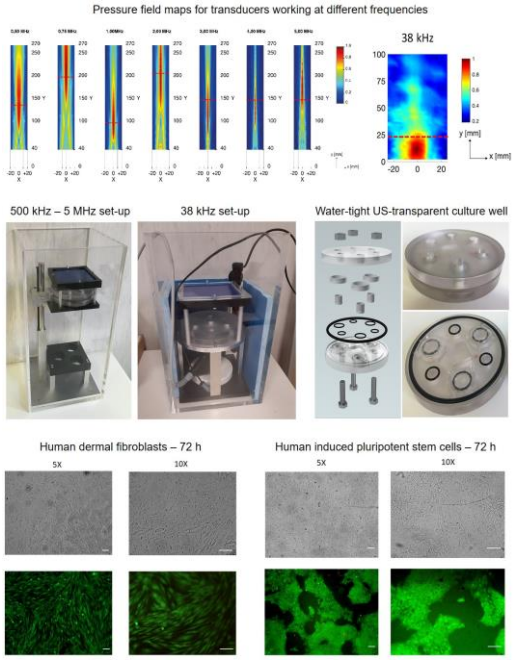

Figure 1. Normalized pressure maps of tranducers working at different frequencies, LIPUS stimulation set-ups and its components and biocompatibility results showing a good cell response to the system (scale bar = 100 μm).

So, similarly to what happens in loudspeakers, piezo nanomaterials generate local electrical charges when invested by US waves. We exploited this effect at intracellular level, by using piezoelectric nanomaterials internalized within cells and then stimulated through LIPUS. This produced an electrical stimulation regime within the cells, which showed clear beneficial regenerative effects on skeletal muscle precursors and other cell types. We also explored the inclusion of piezoelectric nanomaterials within polymeric matrices, thus to fabricate smart responsive nanocomposites to be stimulated through US waves. This also triggered interesting beneficial effects on different cell types, *e.g.* on adipose tissue-derived stem cells, paving the way towards the application of piezoelectric nanomaterials and LIPUS as an innovative treatment strategy for osteoarthritis.

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