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Stimulation of bone repair with ultrasound: A review of the possible mechanic effects

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ABSTRACT

In vivo and *in vitro* studies have demonstrated the positive role that ultrasound can play in the enhancement of fracture healing or in the reactivation of a failed healing process. We review the several options available for the use of ultrasound in this context, either to induce a direct physical effect (LIPUS, shock waves), to deliver bioactive molecules such as growth factors, or to transfect cells with osteogenic plasmids; with a main focus on LIPUS (or Low Intensity Pulsed Ultrasound) as it is the most widespread and studied technique. The biological response to LIPUS is complex as numerous cell types respond to this stimulus involving several pathways. Known to-date mechanotransduction pathways involved in cell responses include MAPK and other kinases signaling pathways, gap-junctional intercellular communication, up-regulation and clustering of integrins, involvement of the COX-2/PGE2, iNOS/NO pathways and activation of ATI mechanoreceptor. The mechanisms by which ultrasound can trigger these effects remain intriguing. Possible mechanisms include direct and indirect mechanical effects like acoustic radiation force, acoustic streaming, and propagation of surface waves, fluid-flow induced circulation and redistribution of nutrients, oxygen and signaling molecules. Effects caused by the transformation of acoustic wave energy into heat can usually be neglected, but heating of the transducer may have a potential impact on the stimulation in some *in-vitro* systems, depending on the coupling conditions. Cavitation cannot occur at the pressure levels delivered by LIPUS. *In-vitro* studies, although not appropriate to identify the overall biological effects, are of great interest to study specific mechanisms of action. The diversity of current experimental set-ups however renders this analysis very complex, as phenomena such as transducer heating, inhomogeneities of the sound intensity in the near field, resonances in the transmission and reflection through the culture dish walls and the formation of standing waves will greatly affect the local type and amplitude of the stimulus exerted on the cells. A future engineering challenge is therefore the design of dedicated experimental set-ups, in which the different mechanical phenomena induced by ultrasound can be controlled. This is a prerequisite to evaluate the biological effects of the different phenomena with respect to particular parameters, like intensity, frequency, or duty cycle. By relating the variations of these parameters to the induced physical effects and to the biological responses, it will become possible to derive an 'acoustic dose' and propose a quantification and cross-calibration of the different experimental systems. Improvements in bone healing management will probably also come from a combination of ultrasound with a 'biologic' components, e.g. growth factors, scaffolds, gene therapies, or drug delivery vehicles, the effects of which being potentiated by the ultrasound.

1. Introduction

There are several ways by which ultrasound can influence bone fracture healing. Ultrasound has played, or has the potential to play, a role in different aspects of the process of bone regeneration. It can act on the biologics components of the regeneration process via promotion of cell proliferation, cells pre-conditioning to orient their differentiation during culture [1,2], or cells transfection [3]. Ultrasound can modulate the micro-environment by triggering delivery of growth factors or gene expression in engineered cells [4,5]; or by modulating the physical environment by heat deposition or mechanical stimulation [6]. Ultrasound can also be useful in tissue engineering approaches by acting on the scaffolds for improvements of scaffold integration, characterization and control

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of the rate of scaffold degradation [7–10]. Within this arsenal, the LIPUS (or Low Intensity Pulsed Ultrasound) techniques aim at modulating the physical environment of the cells, in particular by mechanical stimulation.

Different forms of ultrasound treatment (LIPUS, Shock Waves) have been proposed to stimulate or induce bone repair. Biophysical effects of ultrasound, and in particular of therapeutic ultrasound used for thermal ablation or drug delivery, have been fairly documented [11]. However, the mechanisms by which ultrasound can interact with cells and/or their microenvironments during fracture healing are still open to debate.

Clinical results obtained with ultrasound stimulation of bone healing are still controversial, suggesting a potential effective role but depending of the medical history of previous treatments, site and type of fracture or bone loss (like bone lengthening), pathology (fresh fracture vs. delayed unions) and treatment modality (treatment daily duration, intensity, frequency, etc.), suggesting the need for standardization of treatment dose and for further randomized controlled trials [12,1,13,14]. Moreover, the lack of understanding of the relevant mechanisms that triggers a positive biological response suggests that optimization of devices' technology and treatment regimen remains to be fulfilled.

The main purpose of this review is to give the reader a general idea on existing ultrasound applications for the stimulation of bone healing and treatment of non-unions. The main focus is placed on the LIPUS technique, which has pronounced bioeffects on tissues regeneration, while employing intensities within a diagnostic range [1,13,15]. The updated state of the LIPUS biological knowledge is summarized and discussed through the prism of plausible physical effects implicated with observed biological phenomena.

2. Basics of Biomedical Ultrasound

The term ultrasound refers to the propagation of an acoustic wave, *i.e.* a travelling mechanical perturbation, whose frequency is above the audible range, typically from a few tenth of kHz to several tenths of MHz. Ultrasound in liquid and in soft tissues usually refers to the propagation of a longitudinal wave, causing locallyoscillatory motions of particles around their initial positions. This will result in local changes of the medium's density and pressure, an increase in location of rarefraction (low pressure) and increase in the location of compression (high pressure) cycles of the wave. Depending on the frequency, level of acoustical energy and /or pressure emitted by the source, applications can be categorized as diagnostic or therapeutic, where diagnostic intensities (spatially and temporarily averaged) are typically below 100 mW/cm^2.

Diagnostic applications include gray-scale imaging or 'B-mode' imaging or echography, where the variations in the amplitude of the backscattered ultrasonic waves by tissues are displayed as a function of depth to visualize morphologic features or to analyze structure of the tissues [16]; and elastography for direct imaging of the strain and Young's or shear modulus of tissues, based on a ultrasonic measure of internal tissue motions as a results of the application of a mechanical stimulus [17]. Even if diagnostic ultrasound can potentially induce some biological effects [18], in particular in the presence of ultrasound contrast agents [19], indices like the mechanical and thermal indexes are used to stay within a range of energy/intensity deposition that will avoid them. These imaging technologies can have applications in the characterization of the bone healing process [20,21]. In bone tissue engineering, elastography has also been proposed as a way to monitor scaffold degradation [22].

In contrary to diagnostic applications, therapeutic ultrasound is purposely looking for the induction of bio-effects in tissues. There are several ways by which ultrasound can interact with tissues to induce biological effects [23,24], relying on thermal effects for heat deposition, or non-thermal effects like cavitation or radiation force.

Ultrasound energy absorption is used to elevate temperature in tissues, and high intensity focused ultrasound or HIFU is used to thermally ablate cancer tumors [25,26]. In a more moderate regime, ultrasound energy delivery can also be used to induce mild hyperthermia and is used in physiotherapy to promote healing of both bone and soft tissues [27]. Heat deposition can also be used to control the expression of reporter genes under transcriptional control of a heat-inducible promoter [28]. In shock wave lithotripsy, shock waves, generated outside the body, are focused to a fixed location to produce locally very large acoustic pressures, inducing kidney stones comminution [29]. Ultrasound can also induce temporary cell membrane permeability, a phenomenon called sonoporation [30], and this approach can be used to enhance drug or genetic material uptake [31]. For these effects, the principle mechanism is believed to be cavitation, the growth, oscillation, and eventually collapse of gas bubbles in liquid driven by an ultrasound wave. Combined with thermo- or mechanical-sensitive carriers, like thermo-sensitive liposomes [32], echogenic liposomes [33] or superheated perfluorocarbone droplets [34], ultrasound can be used to control spatially the release of drugs by inducing rupture or pore-like defects in carriers membranes. DNA delivery into various cells *in vitro* and *in vivo* has also been reported [35], demonstrating increased gene expression, even if optimization is still required to achieve high transfection rates [36]. Other therapeutic applications of ultrasound have been developed, such as sonothrombolysis, or ultrasound cutting but are so far not used in applications related to tissue engineering. The reader will find some insight in the review papers [23,37–39].

In between the diagnostic and therapeutic regimes lies the so-called low-intensity pulsed ultrasound technique or LIPUS, where low ultrasound intensity, typically below 100 mW/cm^2, *i.e.* at the upper limit of diagnostic intensities, is delivered in a pulsed manner. In contrast to a diagnostic application, the ultrasound is delivered in a pulsed manner with long duty cycles and exposure times. LIPUS have been reported to be responsible for several biological reactions *in vitro* and *in vivo*, in particular enhancing the healing rate of bone fractures [1] and soft tissue healing [40], with reported cellular responses that we will analyze later in this review.

3. LIPUS physics

3.1. LIPUS exposure conditions

Heating, cavitation and acoustic streaming have been proposed to be the main physical mechanisms to stimulate cells *in vitro*. LIPUS stimulation studies have been conducted with frequencies between 45 kHz and 3 MHz, intensity levels between 5 and 1000 mW/cm^2 (SATA: spatial average, time average), in continuous or burst mode, and with daily exposure times between 1 and 20 min.

The vast majority of the published studies were performed with devices similar to the commercial system Exogen (SAFHS, Exogen, NJ). This system uses unfocused circular transducers with effective surface areas of 3.88 cm^2 and the following typical stimulation conditions: frequency 1.5 MHz, intensity 30 mW/cm^2 (SATA), burst mode 200 µs ON/800 µs OFF (i.e. pulse repetition rate 1 kHz), daily exposure: 20 min [41]. Other studies report the use of unfocused transducers with different surface areas, ultrasound frequencies between 45 kHz [42] and 3 MHz [43], intensity levels between 5 and 2400 mW/cm^2 [42,44], and duty cycles (e.g. 2 ms ON at 100 Hz pulse repetition rate, or continuous wave mode) [43].

For lossless linear plane wave propagation the relation between acoustic impedance $Z = \rho c$, particle velocity v and acoustic pressure P is:

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