Chapter 2

Acoustic Therapy as Mechanical Stimulation of Osteogenesis

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Additional information is available at the end of the chapter

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Abstract

Acoustic therapy is a branch of mechanotherapy. This modality of treatment can be used for osteogenesis-related orthopaedic disorders. Because bone cells are responsive to acoustic forces, specially designed devices were developed to generate acoustic forces in the form of low-intensity pulsed ultrasound, extracorporeal shock waves or radial pressure waves. With the developed devices, it became possible to provide patients an alternative, or adjunctive, treatment for pathologies involving bone homeostasis, that is, the balance of bone formation and bone resorption. The so-called acoustic therapy (low-intensity pulsed ultrasound stimulation, LIPUS; extracorporeal shock wave therapy, ESWT; and radial pressure wave therapy, RPWT) acts through physical phenomena produced when acoustic waves are transmitted into living tissue and converted to biological reactions, thereby activating signalling pathways that drive a cellular response in favour of osteogenesis. In this chapter, an extensive review of the literature was performed to provide the reader the "state of the art" about the physical phenomena, molecular events and clinical uses of acoustic forces for osteogenesis-related orthopaedics disorders.

Keywords: osteogenesis, low-intensity pulsed ultrasound stimulation (LIPUS), extracorporeal shock wave therapy (ESWT), radial pressure wave therapy (RPWT), acoustic forces, mechanotransduction, mechanical loading

1. Introduction

Mechanical stimulation of bone cells modulates a myriad of molecular signalling pathways involved in osteogenesis. There are distinct forms of mechanical forces, such as centrifuge force,

gravitational force, electromagnetic force, hydrostatic force and acoustic force. Acoustic forces comprise a modality of mechanical load that can be represented basically by three different types of acoustic waves: ultrasound wave, shock wave and radial pressure wave. Those waves may be applied to patients suffering from orthopaedics disorders, especially those related to osteogenesis; for instance, delayed union, nonunion, osteoporosis and acute fractures.

The application of mechanical devices for medical purposes is termed mechanotherapy. Accordingly, the use of acoustic devices, which is a category of mechanical devices, for medical purposes will be termed here acoustic therapy and will be further divided into three subcategories: low-intensity pulsed ultrasound stimulation (LIPUS), extracorporeal shock wave therapy (ESWT) and radial pressure wave therapy (RPWT). This chapter discusses the physical phenomena, biological events and clinical indications of acoustic therapy on bone tissue (**Table 1**).

Abbreviations	Meanings	Abbreviations	Meanings
ActR	activin receptor	MCP	monocyte chemoattractant protein
ALP	alkaline phosphatase	MIP	macrophage-inflammatory protein
AT1	angiotensin II type 1 receptor	Msx	Msh homeobox
ATP	adenosine triphosphate	mTOR	mechanistic target of rapamycin
Bax	Bcl-2-associated X protein	NADPH	nicotinamide adenine dinucleotide phosphate
BMP	bone morphogenetic protein	NO	nitric oxide
BMPR	bone morphogenetic protein receptor	NOS	nitric oxide synthase
Ca^{2+}	calcium ion	OPG	osteoprotegerin
cbfa	core binding factor subunit alpha-1, also known as Runx2	PGE_2	prostaglandin E_2
CDK	cyclin-dependent kinase	PPARγ2	peroxisome proliferator-activated receptor γ2
c-fos	FBJ murine osteosarcoma viral oncogene homolog	PTHr	parathyroid hormone receptor
c-jun	Jun proto-oncogene	Rac	Ras-related C3 botulinum toxin substrate
c-myc	avian myelocytomatosis viral oncogene homolog	RANK	receptor activator of nuclear factor kappa B
cox	cyclooxygenase	RANKL	receptor activator of nuclear factor kappa B ligand
CXCR	C-X-C chemokine receptor	RANTES	regulated upon activation, normal T-cell-expressed and secreted
Dlx	distal-less homeobox	Ras	portmanteau of “rat” and “sarcoma”
egr	early growth response	rhBMP-2	recombinant human BMP-2

Abbreviations	Meanings	Abbreviations	Meanings
ERK	extracellular signal-regulated kinase	RPWT	radial pressure wave therapy
ESWT	extracorporeal shock wave therapy	Runx	runt-related transcription factor
FAK	focal adhesion kinase	SDF	stromal cell-derived factor
FGF	fibroblast growth factor	Smad	portmanteau of "small body size" and "decapentaplegic"
HIF	hypoxia-inducible factor	SOST	sclerostin gene
IGF	insulin-like growth factor	TCF/LEF	T-cell factor/Lymphoid enhancer binding factor
IL-8	interleukin-8	TGF	transforming growth factor
ILK	integrin-linked kinase	TSC	transforming growth factor-beta-stimulated clone
IRS	insulin receptor substrate	Tyr	tyrosine residue
LIPUS	low-intensity pulsed ultrasound stimulation	VEGF	vascular endothelial growth factor
LRP	low-density lipoprotein receptor-related protein	Wnt	wingless-related integration site
MAPK	mitogen-activated protein kinase		

Table 1. Abbreviations used throughout text.

2. From concepts to acoustic devices

Bone modelling refers to changes in bone structure and density in response to increased loads. Bone remodelling is defined as, the almost obligatory, bone resorption that follows bone formation irrespective of mechanical loads. The first to describe that bone deposition occurs preferably on sites of compressive loads, whereas bone resorption occurs preferably on sites of tensile loads was Julius Wolff, whose observations were the foundation of Wolff's law (1892) [1].

Later, Frost realized that different ranges of intensities (magnitudes) of bone deformation elicited different biological responses. Based on that, he published the mechanostat model (1964), in which low-frequency cyclic (less than 5–10 Hz), or static, loads lower than 50–100 μstrain (desuse range) lead to bone resorption, loads from 50–100 to 1000–1500 μstrain (physiological range) do not change bone mass, loads from 1000–1500 to 3000 μstrain (overuse range) induce osteogenesis and loads greater than 3000 μstrain (pathological overuse range) may lead to fracture or stress fracture. Strain stands for relative deformation of a cell or tissue. It should be noted that the ranges of intensities proposed by Frost refer to bone tissue, not to bone cells, which normally need higher strains to elicit an osteogenic response [1–3]. For detailed information, see reference [3].

Based on Wolff's law and mechanostat model concepts, mechanical devices were developed to purposely stimulate osteogenesis. LIPUS and ESWT are applied by acoustic devices approved in many countries for clinical use in the management of bone healing disorders. On the other hand, currently, RPWT lacks evidence for its use to induce osteogenesis, but it is used to address soft tissue orthopaedic disorders.

2.1. LIPUS device

Low-intensity pulsed ultrasound was developed by Duarte, and its use for accelerating fracture healing was published in 1983. Most commonly used device generates 1.5 MHz ultrasound in a pulse wave mode (duty cycle of 20%, 200 μs burst width with repetitive frequency of 1 KHz) and average intensity 30 mW/cm^2. Low-intensity ultrasound waves are produced from a piezoelectric crystal within an unfocused, circular transducer. Its effective radiating area is 3.88 cm^2, peak rarefactional pressure at specimen (nonderated) is 0.076 MPa and focal length is ~130 mm [4–6].

2.2. ESWT devices

ESWT originally was developed for lithotripsy in order to break up and disrupt stones within genitourinary tract. Its use for osteogenesis initiated after the observation that shock waves provoked osteogenic response on the pelvis of animals during lithotripsy experiments [7].

There are three main techniques for generation of shock waves. Irrespective of the technique, production of shock waves requires the conversion of electrical energy into acoustic energy. All three devices (electrohydraulic, electromagnetic and piezoelectric) are used in orthopaedics, and there is no evidence that a certain device provides better results than the other [7–10].

2.2.1. Electrohydraulic device

This is the first generation of orthopaedic shock wave devices. A high-voltage electrical discharge is applied across electrode tips—a spark gap—within a water-filled semi-ellipsoid reflector. The resultant spark heats and vaporizes the surrounding water, which, in turn, generates a gas bubble filled with water vapour that expands and produces a shock wave. The wave is reflected by the metallic surface of the semi-ellipsoid and is focused into the therapeutic zone [7, 9, 11].

Electrohydraulic shock wave devices usually are characterized by relatively large axial diameters of the focal volume and high total energy within that volume. The spark gap wears out after about 50,000 shots (impulses) and needs to be replaced [7, 11].

Technical specifications vary according to manufacturer (not all manufacturers provide complete data): energy flux density varies from 0.01 to 1.80 mJ/mm^2, focal zones vary from 0 to 95 mm (fx[-6dB]) and from 4.8 to 25 mm (fz[-6dB]), frequency varies from 0.5 to 360 Hz, and penetration depth is up to 84 mm [12–15].

2.2.2. Electromagnetic device

Within this device, there is an electromagnetic coil and a metal membrane besides the coil both embedded in a water medium. A high current pulse is released through the coil, generating a strong magnetic field, which repels the membrane rapidly away from the coil, therefore pushing the surrounding water to produce a shock wave. The shock wave is focused with an acoustic lens to the therapeutic zone. The lens can be used for several hundred thousand impulses with no need to replace the elements [7, 9, 11].

A variation of the electromagnetic device uses a repelling membrane formed as a cylinder and the sound waves are reflected by a surrounding parabolic reflector [11].

Technical specifications vary according to the manufacturer (not all manufacturers provide complete data): energy density flux varies from 0.01 to 0.55 mJ/mm^2, frequency varies from 1 to 8 Hz shock waves, penetration depth is up to 80 mm and focal zone varies from 0 to 65 mm [16, 17].

2.2.3. Piezoelectric device

Within this device, a few hundred to some thousand piezoelectric crystals—usually more than a thousand—are arranged in a spherical surface filled with water. A high pulse discharge is applied to the crystals, which immediately contract and expand (piezoelectric effect) generating a shock wave in the surrounding fluid. The emitted energy of each crystal is fairly weak, but reaches higher energy at the focus where all shock waves gather together. The focal zone is relatively small and cigar shaped. Because of the spherical shape of the device's surface, this device has an extremely precise focus and a high energy density within a well-confined focal volume. In addition, the large aperture of the source allows for almost pain-free treatment because of the low-pressure at the skin entry zone [7, 9, 11].

Technical specifications vary according to the manufacturer (not all manufacturers provide complete data): energy flux density ranges from 0.03 to 0.4 mJ/mm^2, frequency ranges from 1 to 8 Hz, pressure ranges from 11.5 to 82.2 MPa, focal size ranges from 1.2 to 4.8 mm (fx[-6dB] = fy[-6dB]) and from 1.2 to 14.1 mm (fz[-6dB]) and penetration depth ranges from 5 to 40 mm [18–20].

2.2.4. RPWT

Radial pressure waves are produced pneumatically (ballistically). A projectile is accelerated with compressed air, or an electromagnetic field, within a guiding tube (cylindrical piston) and strikes a metal applicator placed on the patient's skin. The projectile produces stress waves in the applicator that transforms their kinetic energy into a radially expanding pressure—or pulse—wave towards tissue [21, 22].

Technical specifications vary according to model and manufacturer (not all manufacturers provide complete data): energy density flux is up to 0.55 mJ/mm^2, frequency ranges from 1 to 22 Hz, pressure ranges from 1.0 to 5.0 bar and penetration depth is up to 60 mm [23–26].

3. Physical phenomena elicited by acoustic waves in biological tissues

3.1. Forms of acoustic waves

Low-intensity pulsed ultrasound, shock waves and radial pressure waves are different forms of acoustic waves. Their distinct physical parameters are expected to produce different physical phenomena when transmitted into biological tissues.

3.1.1. Low-intensity pulsed ultrasound

Sound is the vibration (rapid motion) of molecules within a compressible medium such as air or water. It can only propagate in compressible media. When sound waves (acoustic waves) reach molecules, molecules may get closer—compression—or farther—rarefaction. By alternating compression and rarefaction, sound travels in waves transporting energy from one location (transmitter) to another (receiver). Because sound waves produce mechanical motion of molecules, they are mechanical waves. When the frequency of a sound wave is above the typical human audible range (greater than 20 kHz), this sound wave is called ultrasound. Ultrasound is an acoustic radiation that can be transmitted as high-frequency pressure waves (1–12 MHz) [4, 6, 7, 27, 28].

Spatial average temporal average (ISATA) lower than 150 mW/cm^2 is generally regarded as the intensity spectrum of LIPUS. ISATA refers to the spatial average intensity over both the on time and the off time of the pulse. Nevertheless, there is no clear-cut upper intensity boundary to define an ultrasound wave as low-intensity ultrasound. LIPUS studies have been conducted with intensity level between 5 and 1000 mW/cm^2, with frequency between 45 kHz and 3 MHz, in continuous or burst mode and with daily exposure times between 1 and 20 min. In spite of that, most used parameters for LIPUS are as originally described by its creator: intensity of 30 mW/cm^2, frequency of 1.5 MHz, pulse (burst mode) of 1 KHz with duty cycle of 20% and daily exposure times of 20 min [4, 29, 30].

3.1.2. Shock waves

They are also acoustic pressure waves, or sonic pulses. In general, a shock wave can be described as a single pulse with a wide frequency range up to 20 MHz (typically in the range from 16 Hz to 20 MHz), high positive pressure amplitude up to 120 MPa (often 50–80 MPa), low tensile wave up to 10 MPa with short duration (about 1 μs), small pulse width at -6dB, short life cycle of approximately 10 μs and a short rise time of the positive pressure amplitude (lower than 10 ns). The reader may find studies with measured rise times of shock wave devices in the range of 30 ns as a result of the limited time resolution of piezoelectric hydrophones. However, optical hydrophones, which are more sensitive measure devices, displayed measure rise times below 10 ns for electrohydraulic devices [7, 9, 22].

The energy density (maximum amount of acoustical energy transmitted through an area per pulse) of ESWT is up to 1.5 mJ/mm^2 and the pulse energy (sum of all energy densities across the beam profile multiplied by the area of the beam profile) is up to 100 mJ. Arbitrarily, energy levels up to 0.08–0.12 mJ/mm^2 in the focal zone are defined as low-energy ESWT, energy levels

between 0.08 and 0.28 mJ/mm^2 are defined as medium-energy ESWT and energy levels greater than 0.28 mJ/mm^2 are defined as high-energy ESWT (some authors consider 0.12 mJ/mm^2 the cut-off from low- to high-energy ESWT) [7–9, 22, 31, 32].

3.1.3. Ultrasound vs shock waves

Shock waves differ from regular sound waves in that the wave front, where compression takes place, is a region of sudden change in stress and density. Shock waves travel faster than sound, and their speed increases as the amplitude (pressure) is raised. On the other hand, the intensity of a shock wave decreases faster than does of a sound wave. As a consequence, wavelets at high pressure lead to deformation of the wave so that the wave crest assumes a sawtooth appearance, which is different from the sinusoidal appearance of a regular sound wave. Furthermore, shock waves differ from ultrasound waves since the former is uniphasic with high peak pressure (in the order of a hundred MPa), and the latter is biphasic with very low peak pressure (in the order of a hundredth of MPa) [7, 22].

3.1.4. Radial pressure waves

Considering the physical definition of shock waves, radial pressure waves are wrongly termed unfocused shock waves in the literature. The rise time of the positive pressure waves produced by currently available devices are much greater than 10 ns, varying from 600 to 800 ns. Also, the maximum peak positive pressure of a radial pressure wave device varies from 0.1 to 7 Mpa, and the pulse duration varies from 1 to 5 ms. Since the time taken for the radial wave to rise is too long, the curve of the concave surface of the ray is too wide for it to be possible to focus the energy; therefore, radial waves cannot be focused, unlike ESWT. Moreover, the air pressure-accelerated projectile has a speed from 2 to 20 m/s, which is 2 orders of magnitude slower than sound speed in water or tissue. Shock waves are produced when the projectile speed is comparable or higher than sound speed (i.e. supersonic). In addition, the distinction between RPWT as "low-energy therapy" and ESWT as "high-energy therapy" is not correct. Most protocols of RPWT use energy density lower than 0.20 mJ/mm^2, but the device can reach up to 0.55 mJ/mm^2. Accordingly, ESWT has a wide range of energy density protocols varying from 0.02 mJ/mm^2 to more than 0.60 mJ/mm^2 [8, 21, 22, 26, 33].

3.2. Attenuation of acoustic energy

When an acoustic wave is transmitted into a biological tissue, a portion of the acoustic energy is reflected, another portion is attenuated (lost) and the other portion is refracted and continues propagating. Much from the attenuated portion is absorbed by irreversible conversion of acoustical energy into heat mainly via viscous friction, and less is scattered by inhomogeneities within tissue that redirect some sonic energy to regions outside the original wave-propagation path. If the density of the inhomogeneity is high, multiple scattering may occur. Therefore, acoustic energy may scatter several times until it is completely absorbed by tissue and converted to heat [27, 34, 35].

Bone has one of the highest attenuation coefficients among biologic tissues. Besides, as frequency increases, penetration decreases and attenuation increases. Therefore, acoustic waves tend to produce heat preferentially in bones and joints. Accordingly, tissue damage and pain may be produced if the intensity of acoustic energy is high enough. For instance, continuous unfocused ultrasound waves in the range of 4000–5000 mW/cm^2 at 1 MHz for 5 min increase temperature by 1.8–4.3°C at different areas of bone within 1–3 cm of distance. On the other hand, ultrasound at intensities of 20–50 mW/cm^2, which is LIPUS, produces negligible variation of tissue temperature (0.01 ± 0.005°C). Moreover, reports using very high ultrasound intensities (5000–25,000 mW/cm^2) showed delayed bone healing and necrosis, whereas ultrasound at intensities of 200–3000 mW/cm^2 has been shown to increase callus formation and accelerate fracture healing [4, 27, 35–38].

Extracorporeal shock waves and radial pressure waves also increase temperature of tissues either by absorption or by cavitation (see Section 3.3). However, no reports were found about temperature raise within biological tissues subjected to ESWT and RPWT. In spite of that, thermal effects may be responsible for decreased cell viability immediately after ESWT with some energy densities and number of impulses [39–44].

3.3. Cavitation bubbles

When near gas or vapour bubbles, a portion of the refracted acoustic wave may generate cavitation bubbles at locations termed "nucleation sites". Cavitation refers to a range of complex phenomena that involve the creation, oscillation, growth and collapse of bubbles within a liquid or liquid-like medium. Cavitation bubbles have never been confirmed in living tissues; therefore, the following information is based on mathematical simulations and in vitro studies [3, 27, 37].

The occurrence and behaviour of cavitation depend on the acoustic pressure; the existence of microheterogeneities in liquids such as free gas, solid particles or a combination of both; whether the acoustic field is focused or unfocused, or pulsed or continuous; and the nature and state of the material and its boundaries. Cavitation does not occur with ultrasound intensities below 500 mW/cm^2. Consequently, LIPUS does not produce the phenomenon of cavitation. On the other hand, the biological effects of ESWT and RPWT are triggered mainly by the phenomenon of cavitation [4, 27, 30, 38, 45].

Bubbles are gas-filled spheres in a liquid under constant hydrostatic pressure when there are no acoustic waves. In response to a sound field in which the acoustic pressure varies sinusoidally in time with a given frequency, the bubble radius oscillates (expands and contracts) with radial displacement and velocity, which vary sinusoidally in time with the same frequency of the wave. When there is lower level pressure amplitude in synchrony with bubble motion, the immediately surrounding liquid moves in and out creating a small steady flow of fluid called microstreaming. This is called stable cavitation and may occur with low-energy ESWT and some RPWT. Stable cavitation occurs near a solid boundary (e.g. bone) and creates shear stress near the bubble surface that can also mechanically stimulate cells [7, 45].

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Shock waves and radial pressure waves generate cavitation bubbles during the tensile phase of the acoustic wave due to its tensile forces that exceed the dynamic tensile strength of water. During the growth phase of the bubble, a huge amount of energy is delivered to the bubble. Following a number of shock, or radial pressure, wave pulses (sometimes after the first impulse), the bubble collapses (i.e. experiences an extremely rapid contraction), which is called inertial cavitation. As the bubble collapses, four phenomena can be observed [7, 27, 35, 45, 46]:

1. Release of energy in the form of high temperature, which can produce free radicals that may damage cells. However, the production of free radicals has not been confirmed in living tissue.
2. Secondary shock wave emission into the fluid that produces a direct mechanical effect on tissue.
3. The bubble may aggregate with surrounding bubbles, may fragment or may repeat the growth/collapse cycle several times.
4. When bubble collapse is not perfectly symmetric, a liquid jet can form. The liquid jet traverses the bubble and impacts on the surface of tissue perpendicularly at considerable speed.

Additionally, during the positive pressure phase of a second shock, or radial pressure, wave pulse, may also push the liquid of the surrounding medium towards one of the walls of a preformed bubble. That wall goes under deformation and reaches the opposite wall of the same bubble to originate a water jet in the same direction of the propagation of the shock wave. The formation of a water jet usually occurs in the vicinity of boundary areas between materials of differing density, such as bone and cartilage, in the direction of the boundary area. The generated water jet is faster with increasing softness of the interface and more damaging than jets from inertial cavitation. In addition, the presence of a hard biomaterial (e.g. bone and cartilage) causes the bubble to collapse towards it. Besides, as the bubble expands, the interface between the medium and the biomaterial is pushed away from the bubble; however, when the bubble collapses, the interface moves slightly towards the bubble. It should be noted that inertial cavitation bubbles near softer material, such as fat, skin and muscle, tend to collapse by splitting into two or three smaller bubbles without the formation of water jets [7, 21, 38, 46, 47].

3.4. Acoustic radiation pressure

This is the proposed mechanism by which LIPUS stimulates living tissues. The authors also believe this is the main mechanism by which ESWT and RPWT stimulate living tissues. Acoustic radiation pressure tends to increase in proportion to intensity, is generally relatively small in magnitude and produces forces and motions at much lower frequencies than those of the incident acoustic wave. While the tensile phase of the shock and radial pressure waves generates cavitations, the positive pressure phase of those waves produces acoustic radiation pressure [9, 35, 37, 45].

Radiation pressure is a universal phenomenon in any wave motion involving sound. It is exerted on surface or media interfaces and acts in the direction of propagation of the wave thereby producing direct and indirect mechanical stress. Direct mechanical stress is produced by strain. Following mechanical deformation, bone exhibits electrical activity and cellular activation. It is unclear, however, whether the main responsible for bone electrical activity is piezoelectricity, streaming potentials, or ion channels and ATP receptor activities (see 3.4.1) [4, 28, 34, 45].

Indirect mechanical stress is produced by acoustic streaming and modal conversion. When acoustic waves are refracted from water to soft tissues, waves propagate longitudinally (in the same direction of the beam source) due to impedance similarity. Differently, when acoustic waves are refracted to materials with impedance mismatch, such as bones, modal conversion occurs, that is, shear waves (waves at right angles to the direction of the beam source) are produced along with longitudinal waves. Shear waves may produce direct mechanical deformation to tissue, called shear stress [7, 27, 36, 45].

Acoustic radiation pressure decreases with the distance of the wave from its source; hence, radiation pressure gradients are formed within the fluid. As a result, fluid flow originates, which is called acoustic streaming. The flow is directed away from the transducer with gradual build up of the axial streaming speed with distance from the transducer and a peak of velocity in the focal region. The fluid flow continues beyond the focal region and returns to the transducer as recirculation vortices. Fluid flow can also build up again in the acoustic beam after a membrane. Acoustic streaming and microstreaming are often used as synonyms in the literature. Although both produce fluid flow which can modulate osteogenesis, they are distinct phenomena. As described above, acoustic streaming results from radiation pressure gradients, whereas microsteaming is generated by stable cavitation bubbles. Furthermore, not only mechanical deformation but also acoustic streaming increases cell membrane permeability and generates streaming potentials. It has not been shown, however, whether acoustic streaming directly affects cell membrane permeability, or triggers cellular reactions that increase membrane permeability [30, 37, 45, 48]. For a detailed explanation of streaming potentials, see reference [3].

3.4.1. Electric potentials

Bone exhibits electrical activity when subjected to mechanical forces. The opposite is also true: bone undergoes deformation when exposed to electric potentials. For instance, ESWT induces transient cell membrane hyperpolarization. There are three possible contributors to electric potentials on bone. First, mechanically induced activity of ion channels and ATP receptors promotes ion transport between the intra and extracellular environments resulting in membrane action potentials; second, piezoelectricity, which is the generation of electricity when asymmetric crystalline materials—as those that form the extracellular matrix of bone—are subjected to strain; and finally, streaming potentials that result from mechanically induced flow of fluid containing high conductivity ions [3, 4, 44, 49].

4. Mechanosensation and mechanotransduction

4.1. Strain amplification

The various cell types that populate bone—osteoblasts, osteocytes, osteoclasts, periosteal cells (fibroblasts and progenitor cells) and bone marrow cells (include mesenchymal stem cells)—are responsive to mechanical stimulation. Bone is a hard material that can handle up to 2% of strain (i.e. 20,000 μstrain) without failure (fracture). However, based on in vitro studies, bone cells need strains up to 10% in order to direct their response to osteogenesis. In addition, a large amount of energy is lost during wave propagation within bone by means of attenuation and reflection; as such, bone cells may be exposed to low pressure waves. A possible explanation would be that strains are amplified at tissue level, so that cells are exposed to higher strain intensities. At the moment, that hypothesis could not be proved. Nevertheless, the following mathematic-based model supports that explanation [1–3, 41, 43, 44, 50–57].

The model for strain amplification was based on the microanatomy of osteocytes, which are the main mechanosensors of bone. Cytoplasmic processes of osteocytes are separated from their canalicular wall by a pericellular space filled with albumin-rich fluid. Moreover, cytoplasmic processes are anchored to their canalicular wall by transverse fibrils. When mechanically induced fluid flow collides with fibrils, hoop strains are generated on the membrane-cytoskeleton system of cytoplasmic processes. Hoop strains produce forces which are 20–100 times higher than at bone's surface. The magnitude of hoop strains depends on the relationship between fluid and transverse fibrils within pericellular space, and between cell membrane and cytoskeleton constituents (i.e. actin filaments and fimbrins) [3, 57–61].

4.2. Mechanoreceptors

Several structures at cell membrane act as "mechanoreceptors." Mechanically-induced structural deformation of mechanoreceptors triggers their activation. Sequentially, a cascade of biological reactions initiates and results in osteogenesis. Known mechanoreceptors of bone cells include integrins, ATP receptors, ion channels, growth factors (includes hormones) receptors, low-density lipoprotein receptors, frizzled proteins, G proteins and connexins. Among those mechanoreceptors, only integrins were proved to have a role in mechanosensation of ESWT. Regarding mechanosensation of LIPUS, integrins, ATP receptors, growth factors receptors, low-density lipoprotein receptors and frizzled proteins have established participation. In the following sections, the molecular events triggered by LIPUS and ESWT are described. To date, RPWT effects on bone cells have not been investigated properly. It should be noted that acoustic loading refers only to LIPUS, ESWT or RPWT, and mechanical loading refers to any type of mechanical forces that may, or not, be acoustic loads. **Tables 2** and **3** enlist molecular events related to LIPUS and ESWT [1, 10, 50, 55, 62–66].

4.2.1. Integrins

Mechanoreceptors convert mechanical deformations into biological reactions, a process called mechanotransduction. Among mechanoreceptors, it is believed that integrins are vital for mechanotransduction. Evidence suggests the activation of all others mechanoreceptors and a multitude of signalling pathways are integrin-dependent. Therefore, osteogenic response of bone cells (adhesion, migration, differentiation and proliferation) depends on integrins. The expression of α2, α5, β1, β3 integrins subunits are increased by mechanical loading. Furthermore, clusters of α5β1 and αvβ3 integrins formed at the deformation site—also known as focal adhesions—attract a number of cytoplasmic proteins and trigger a cascade of reactions [3, 10, 37, 43, 50, 53, 55, 60, 67–71].

Signalling pathways	**LIPUS**	**ESWT**
α5β1 and αvβ3 integrins/FAK/Scr/Grb2/Sos/Ras/Raf-1/MEK/ERK/IKKα,β/IκBα/NFκB/cox-2/PGE_2	X	
α5β1 and αvβ3 integrins/FAK/Scr/Grb2/Sos/Ras/Raf-1/MEK/ERK/IKKα,β/IκBα/NFκB/iNOS/NO	X	
α5β1 integrin/FAK/Scr/Grb2/Sos/Ras/Raf/MEK/ERK		X
α5β1 and αvβ3 integrins/FAK/PI3K/Akt/NFκB/cox-2/PGE_2	X	
α5β1 integrin/β-catenin	X	X
AT1/ERK-1,2	X	
Ras/Rac1/NADPH/superoxide/ERK/cbfa1		X
Ras/Rac1/NADPH/superoxide/HIF-1α/VEGF		X
α5β1 and αvβ3 integrins/FAK/PI3K/Akt/Bcl-2	X	

Table 2. Signalling pathways triggered by acoustic stimulation.

Biological effects	**LIPUS**	**ESWT**
Increased expression of α5 and β1 integrins	X	X
Increased expression of α2 and β3 integrins	X	
β1 and β3 integrins clustering	X	
α5β1-mediated FAK activation	X	X
αvβ3-mediated FAK activation	X	
Increased IRS-1 activity	X	
Increased $P2X_7$receptor activation and activation, and ATP release	X	
$P2Y_1$ receptor activation		
mTOR activation	X	
Bax expression	X	X
ILK phosphorylation	X	

Biological effects	LIPUS	ESWT
IκBα degradation	X	
Increased parathyroid hormone receptor-1 expression	X	
Increased iNOS, NO, cox-2 and PGE_2 production	X	X
Increased HIF-1α and VEGF expression	X	X
RANKL production	X	
Increased IGF-1 production	X	
Increased TGF-β1 production		X
Increased cyclin E2/CDK2 activation		X
Increased bone sialoprotein expression	X	X
Increased osterix expression	X	
Increased osteopontin expression	X	X
Increased osteocalcin expression	X	X
Increased ALP activity	X	X
Increased type I collagen expression	X	X
Increased bone nodule formation	X	X
Increased CBFA1 expression (core binding factor alfa-1)	X	X
Increased SDF-1 (serum and bone) and CXCR4 expression	X	
Increased c-fos, c-jun, c-myc, TSC-22 (transforming growth factor-beta stimulated clone), SOST, FGF-23, Msx2, Dlx	X	
Increased BMP-2	X	X
Increased BMP-4, BMP-7, BMPR-IA, BMPR-IB, ActR-I, BMPR-II, ActR-IIA, ActR-IIB, Smad1	X	
Increased FGF-2		X
Increased egr-1 (early growth response)	X	
Decreased PPARγ activity	X	
Increased superoxide production		X
Osteoblast differentiation	X	
Osteoblast proliferation	X	X
Osteoblast adhesion		X
Osteoblast migration		X
Bone marrow cells proliferation	X	X
Bone marrow cells osteogenic differentiation		X
Mesenchymal stem cell migration and differentiation	X	

Table 3. Biological effects of LIPUS and ESWT.

4.2.2. ATP receptors

ATP receptors promote the exchange of calcium from intracellular deposits to extracellular environment, or from extracellular environment to intracellular environment. ATP receptors complex with integrins and G proteins, and some ($P2X_7$ and $P2Y_1$) are activated by mechanical loading. By means that need to be explored, mechanically induced activation of $P2X_7$ and $P2Y_1$ induce osteogenic differentiation—represented by increased expression of cbfa-1, osterix, type I collagen, bone sialoprotein, osteopontin and osteocalcin—and osteoblasts proliferation [3, 64, 72].

4.2.3. Wnt pathways

Activation of Wnt canonical pathways involves the formation of complexes between Wnt1, or Wnt3a, Frizzled proteins and LRP-5/6, which may be integrin dependent. Those complexes prevent cytoplasmic β-catenin degradation, which, in turn, translocates to nucleus, where it activates members of the TCF/LEF family to promote osteogenesis. Acoustic stimulation increases expression of Wnt1, Wnt3a, β-catenin and Frizzled proteins 2/4. It also activates β-catenin in an integrin-dependent manner. Wnt5a, which plays a role in Wnt non-canonical pathway, is responsive to mechanical stimulation, but its responsiveness to acoustic stimulation is yet to be evaluated [3, 10, 73].

4.2.4. Growth factors and hormones crosstalk

Different growth factors and hormones induce osteogenesis that includes bone cells proliferation, migration and adhesion to stimulation sites, angiogenesis and osteogenic differentiation. Mechanical stimulation possesses the same effect and affects growth factors and hormones signalling. Acoustic stimulation increases the expression of BMP-2/4/7 and related receptors (BMPR-IA, BMPR-IB, ActR-I, BMPR-II, ActR-IIA, ActR-IIB), FGF-2, IGF-1, PTHr-1, TGF-β1 and VEGF. Nevertheless, the exact signaling pathways of those factors are still not fully understood [37, 44, 54, 66, 74–80].

BMP-2, BMP-4 and BMP-7 play important roles in osteogenesis following fracture. They stimulate mesenchymal cell proliferation and osteogenic differentiation, induce osteoprogenitor cell migration, modulate osteoclast activity and promote angiogenesis. Their mechanism of action involves Smad-1, which is activated by BMP receptors; then Smad-1 translocates to nucleus where it upregulates transcription of osteogenic factors as cbfa1. Acoustic stimulation activates Smad-1, but it has not been proved whether BMP receptors activity is responsible to Smad-1 acoustically induced activation [66, 75].

Similar to BMPs, TGF-β1 induces cellular proliferation, osteogenic differentiation, mineralization and angiogenesis. Acoustic force-induced TGF-β1 production depends on superoxide production which is possibly promoted by Ras/Rac-1/NADPH oxidase pathway. Superoxide is a free radical that, in contrary to common knowledge, is harmless to bone cells when produced by a certain range of acoustic pressure (that is yet to be determined). Moreover, superoxide promotes ERK activation, which induces osteogenic differentiation through cbfa-1 transcription [79, 81].

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Angiogenesis is vital for fracture healing. BMPs, TGF-β1 and VEGF induce angiogenesis. Among those factors, VEGF seems to be the most important for angiogenesis. HIF-1α is a transcription factor that regulates VEGF expression and is activated by acoustic stimulation. In addition, superoxide and Ras mediate HIF-1α activation and VEGF expression. However, VEGF expression is not dependent on BMP-2, TGF-β1, IGF-1, cox-2, PGE_2 and Ca^{2+} influx. Interestingly, LIPUS-induced VEGF expression depends on NO production, whereas ESWT-induced VEGF expression does not [81–83].

4.2.5. Differentiation markers and transcriptional factors

A variety of differentiation markers are modulated by acoustic stimulation, such as cbfa-1, osterix, bone sialoprotein, osteopontin, osteocalcin, type I collagen and ALP. Contrarily, the unique report investigating RPWT effects in osteoblasts showed decreased expression of cbfa-1, osterix, type I collagen, bone sialoprotein and osteocalcin. RPWT is commonly used for orthopaedic pathologies of soft tissues with satisfactory results, but no reports were found for bone-related orthopaedic disorders. Therefore, further investigations are required to determine the biological effects of RPWT on bone [33, 43, 44, 75, 76].

Regarding cellular proliferation, there are some transcriptional factors that are affected by acoustic forces, such as c-fos, c-jun, c-myc, egr-1, TSC-22, SOST, FGF-23, Dlx, Msx2 and cyclin E2/CDK2 [37, 39, 43, 55, 68, 69, 84–86].

4.2.6. Signalling for migration

Cells must migrate to the healing site so that new bone can be generated. SDF-1 is an important chemotactic factor mostly produced by immature osteoblasts in the endosteal region near stem cells population. SDF-1 normally is released from the fracture site to attract mesenchymal stem cells which will differentiate into osteoblasts. SDF-1 binds to CXCR4, a seven transmembrane G-protein coupled receptor, and triggers a cascade of reactions leading to cellular migration and survival. Reports have shown that acoustic loading increases expression of SDF-1 and CXCR4, thereby resulting in mesenchymal stem cells migration to the fracture site. In spite of that, more investigation is needed to clarify the exact cascade of reactions triggered by SFD-1/CXCR4 [56, 87–89].

4.2.7. Signalling for bone remodelling

Bone remodelling is an important step of bone healing. This important stage follows bone formation and is governed by osteoclasts—bone cells of the granulocyte/monocyte lineage—that resorb extracellular matrix. In order to attract osteoclast progenitor cells to the healing site, osteoblasts express MCP-1, MIP-1, RANTES and IL-8. Osteoblasts also express, or secrete, RANKL, which induces osteoclasts differentiation through their native RANK; and secrete OPG, a decoy receptor of RANKL, which antagonizes RANKL-mediated osteoclastogenesis. Acoustic loading in the form of LIPUS affects osteoclastogenesis by increasing the expression of MCP-1, MIP-1b, RANKL and OPG in osteoblasts through AT1. Increased RANKL expression is also dependent on integrins activity. Moreover, acoustic forces increase the expression

of MIP-2, which may also be involved in osteoclastogenesis. On the other hand, it has been shown that low-energy ESWT decreases OPG and RANKL expression in osteoblasts; RPWT does not change OPG expression, but decreases RANKL; and LIPUS does not change the expression of OPG (contradictory results) and RANTES in osteoblasts. Those data show that acoustic deformation affects osteoclastogenesis; however, the exact influence on osteoclastogenesis needs to be better elucidated [33, 43, 50, 55].

4.2.8. Proteins with few data

There is another list of proteins whose activation has been shown to be influenced by acoustic waves, but there is poor information about their role in mechanotransduction and osteogenesis:

1. AT1 is classically involved in arterial pressure control. This receptor was identified in bone cells, but its role is yet to be determined. AT1 is required for mechanically induced ERK-1/2 activation [50].
2. Bax is a key component for apoptosis induced through interactions with pore proteins on the mitochondrial membrane. Bax mechanism of activation is complex and not fully understood, but may be modulated by acoustic deformation in favour of cell survival. Bax activation is also integrin dependent [43, 70].
3. IRS-1 activity increased in intact and healthy bones of rats subjected to acoustic stimulation. IRS-1 is involved in insulin-mediated and IGF-1-mediated bone formation, but its mechanism of activation following acoustic loading is yet to be determined [90].
4. p38 is a MAPK that regulates cell proliferation and differentiation. Because conflicting results were found for p38 activation following LIPUS and ESWT, more investigation is required. Some studies report increased activity, while others report unchanged activity [68, 81, 91, 92].
5. PPARγ2 is expressed in mesenchymal stem cells. Upon acoustic stimulation, PPARγ2 drives those cells to differentiate into osteogenic lineage [68].

5. Optimization of biological responses

As previously described, acoustic loads can be exerted by different types of waves, such as LIPUS, ESWT and RPWT. Changing some physical properties (e.g. magnitude and frequency) and mode of application (e.g. axial distance, incidence angle and number of cycles) of acoustic waves can elicit different cellular responses. No studies explored the subject with RPWT.

5.1. Magnitude and number of cycles

According to mechanostat model, for strains within the overuse range, bone formation increases as a proportion of the load magnitude. Loads within the pathological overuse range

stimulate osteogenesis, but also damage tissue until bone breaks (about 15,000–20,000 μstrain). Furthermore, cellular response also increases as a proportion of the number of cycles [3].

LIPUS at intensities between 2 and 150 mW/cm^2 were compared. Higher intensities produced greater bone formation, faster healing rate, and better torsional stiffness and failure torque. The best results were found for 30 mW/cm^2. Average temperature at the soft tissue was 1.74°C higher for 150 mW/cm^2 in comparison with 30 mW/cm^2. Temperature elevation may affect some enzymes like collagenase I and cause tissue damage, resulting in worse biological response. LIPUS commonly is applied as a daily 20-min treatment; therefore, the number of cycles are not changed [29, 93, 94].

On the contrary, there is no exact protocol for ESWT that determines the best response to stimulation. ESWT at magnitudes ranging from 0.05 to 0.62 mJ/mm^2 positively affects osteogenesis. The number of impulses of shock waves corresponds to the number of cycles of ESWT. In studies, number of impulses varies from 250 to more than 4000. Moreover, biological response is different when treatment is performed in vitro, or in vivo with small animals (e.g. rodents), or in vivo with large animals (e.g. goats) and humans. For most in vitro studies, 500 impulses promote the best cellular response; above this threshold, cellular damage surpasses bone formation. On the other hand, the best intensity (mJ/mm^2) could not be found, suggesting that, for cells directly exposed to ESWT, the number of cycles affects cellular response more than the intensity of energy density itself. On the other hand, most in vivo studies in animals show that better responses are elicited by higher energy densities up to 0.47 mJ/mm^2 in comparative studies, while number of cycles (impulses) was not proved to have the same influence [54, 95–98].

5.2. Frequency

Normally, load frequencies within the range of 1–30 Hz at physiological and overuse ranges progressively induce osteogenesis. Higher frequencies (17–90 Hz), in the form of vibration, induce osteogenesis but at a much lower strain range (about 5 μstrain; i.e. strain in the order of 10^{-5}). LIPUS is a low magnitude and high frequency wave, which, based on mathematical and experimental models, produces strains in the order of 10^{-5} at 1.5 MHz. Because of high frequency, those strains promote the same effects as strains in the order of 10^{-1} (i.e. 10% = 100,000 μstrain) at 1 Hz on cells, and the estimated intracellular strain on organelles is about 0.5% (i.e. 5000 μstrain). Accordingly, it was shown that LIPUS increased transcriptional factors (c-fos, c-jun and c-myc) as frequency increased, resulting in maximum response at 5 MHz (within a range from 2 to 8 Mhz). Those calculations were obtained for strains at cellular level. For strains at bone level, it is believed that the model for strain amplification (see Section 4.1) may apply firstly, followed by the estimative presented here. No investigations were found about the role of frequency on ESWT and RPWT [3, 99–101].

5.3. Axial distance

Energy distribution varies according to the distance from the transducer and the surface (axial distance). For LIPUS, two zones were defined according to axial distance: near field (close to

transducer) and far field (about 130 mm away from transducer). There is also a mid-near field, when the surface is about 60 mm away from transducer. Within near field, energy distribution of LIPUS beam is not uniform. As such, there are many peaks of acoustic pressure (maxima and minima) across the beam diameter. As the distance from transducer increases, the number of peaks of acoustic pressure across the beam diameter decreases (less maxima and minima). When surface is at far field, a regular beam is formed [5, 102].

As LIPUS transducer is placed transcutaneously during treatment, superficial and deeper cells are exposed to different acoustic fields. Although LIPUS promotes osteogenesis within near, mid-near and far fields, axial distance affects the biological effects of LIPUS. Mid-near field LIPUS elicited greater callus formation in a fractured-femur rat model; on the other hand, in that same model, femurs subjected to far field LIPUS exhibited higher peak torque and torsional stiffness. Those results indicate that, mid-near field LIPUS is optimal for cellular proliferation, while far field LIPUS stands for osteogenic differentiation (bone mineralization). Reinforcing that, mid-near LIPUS incited more NO production whereas far field LIPUS promoted increased ALP activity and mineralization in preosteoblasts. Moreover, both mid-near and far field LIPUS produced increased β-catenin nuclear translocation [5, 102].

During ESWT, maximal intensity of energy density is obtained at the focus. Consequently, superficial and deeper cells are exposed to different acoustic fields. However, no studies were found on this subject for ESWT and RPWT.

5.4. Incidence angle

As previously described, acoustic waves transmitted into bone can be decomposed in longitudinal waves and shear waves. The magnitude of each wave depends on the incidence angle of the acoustic wave. Accordingly, two critical angles were determined. The first critical angle is defined as the angle of incidence after which incident acoustic waves travel along the medium surface and only shear waves are refracted to that medium. In that case, longitudinal waves do not travel into the medium. The second critical angle is defined as the angle at which acoustic waves are totally reflected and shear waves travel along the medium surface, but not into the medium. For LIPUS, the first critical angle is 22°, and the second critical angle is 48°. Between the first and second critical angles, at 35°, the amount of transmitted shear waves is maximized, and an optimal cellular response is obtained. Those critical angles were not determined for ESWT and RPWT [36].

5.5. Different sources of acoustic waves

As previously described, the method for producing low-intensity pulsed ultrasound waves is unique, but there are three generation methods for extracorporeal shock waves. No clinical studies compare the effectiveness between the three methods, but one experimental research compared osteoblasts responses to electrohydraulic and electromagnetic ESWT. It was found greater cell viability and osteocalcin expression for electrohydraulic-stimulated cells, and greater expression of type I procollagen-C enzyme, and TGF-β1 production for electromagnetic-stimulated cells. These findings can be attributed to the difference in the pressure

distribution at the focal zone between the electrohydraulic and electromagnetic generators [40].

5.6. Combined therapy

Mechanical stimulation can be combined with different types of acoustic waves or with growth factors.

5.6.1. ESWT and LIPUS

Electromagnetic ESWT and LIPUS combined therapy applied to periosteal cells showed no difference regarding cell proliferation, cell viability and ALP activity in comparison with ESWT alone, but, in comparison with LIPUS alone, showed worse results for early response (after 6 days) and better results for late response (after 18 days) [42].

5.6.2. LIPUS and growth factors

BMPs are known osteogenic factors. Their combined therapy (BMP-7 or rhBMP-2) with LIPUS enhances bone formation, osteogenic differentiation and biomechanical properties of bone [103, 104].

Bisphosphonates are anti-osteoclastic agents that increase or maintain bone mineral density in osteoporotic patients. Combined therapy with LIPUS is not better than alendronate or LIPUS alone to increase bone healing. On the other hand, combined therapy with LIPUS enhances bone mineral density more than separate treatment [105].

1,25-Dihydroxyvitamin D3 increases the expression of VEGF in osteoblasts and modulates cellular proliferation and differentiation. Combined treatment with LIPUS, however, does not ameliorate cellular response in comparison with LIPUS or 1,25-dihydroxyvitamin D3 alone [106].

Statins (e.g. simvastatin, mevastatin and lovastatin) stimulate osteogenesis through Ras/Smad/ERK/BMP-2 pathway. Combined therapy with LIPUS does not increase bone formation rate more than statins or LIPUS alone [77].

No studies were found on the subject for ESWT and RPWT.

6. Acoustic therapy

Clinical applications for acoustic therapy include nonunions and delayed unions. Debatable applications include acceleration of fracture healing, acceleration of segmental defects healing, enhancement of bone density and quality, management of stress fracture, enhancement of bone-tendon junction healing and management of avascular necrosis of the femoral head.

LIPUS has a unique protocol of treatment, which consists of daily 20-min sessions at 30 mW/cm^2. On the contrary, ESWT has no established protocol regarding energy levels, frequency,

number of sessions and number of cycles (impulses). This heterogeneity makes it difficult for the clinician to adopt the best approach for ESWT. No studies were found on the subject for RPWT.

6.1. Delayed union and nonunion

Normally, patients with nonunion and delayed union are managed surgically for revision of a primary surgery or for biological stimulation. Those managed surgically for biological stimulation may be the best candidates for a non-invasive approach with acoustic therapy, since there is no problem with hardware and fracture reduction. Those experiencing technical problems related to the first procedure (gross bone instability, broken hardware, malalignment) should be subjected to revision surgery combined with acoustic therapy to provide also biological stimulation.

LIPUS exhibits healing rate from 67 to 92% and may challenge surgical treatment for delayed union and nonunion. Patients aged 70–79 years feature decreased healing rates (83.3 vs 86.2%), and older than 80 years feature even lower healing rates (77.8 vs 86.2%). LIPUS may also be an alternative approach to treat conservatively congenital pseudarthrosis of the tibia. Mean body mass index, open fracture, multiple prior surgical procedures, time to initiate treatment with LIPUS, type of surgical procedure, comorbidities and number of smoking years represented no risk factor for failure with LIPUS in a cohort of 767 patients. Smaller cohorts present some conflicting data: decreased healing rate was found in late treated (more than 12 months) nonunions and smokers. Moreover, atrophic nonunions may be a risk factor for decreased healing rates. Interestingly, LIPUS combined with iliac crest autograft exhibits synergistic effect to overcome spinal pseudarthrosis created by nicotine administration, although LIPUS alone cannot [94, 107–114].

ESWT also shows healing rates that may challenge surgical treatment for nonunion and delayed union, with successful rates ranging from 63.6 to 95% using electrohydraulic or electromagnetic devices. No reports explored the effectiveness of piezoelectric devices, and RPWT. Energy density varied from 0.25 to 0.70 mJ/mm^2, 1000–10000 impulses, single or multiple sessions. Technical parameters depended on bone size and authorship. Specifically for scaphoid pseudarthrosis, energy density varied from 0.05 to 0.12 mJ/mm^2 depending on patient's pain tolerance. Some studies also investigated serum level of BMP-2, NO, TGF-β1 and VEGF, which were higher in treated individuals. Again, atrophic nonunions, smoking and treatment performed at late stages (after 12 months) provided decreased healing rates [115–124].

6.2. Accelerated healing of bone defects and fractures

The potential benefits of LIPUS and ESWT to accelerate healing of bone defects and fractures have been shown in various animal studies, but there is not sufficient clinical evidence to support their routine use.

LIPUS promoted earlier callus formation, promoted larger callus width, increased biomechanical strength, reduced adverse outcomes (nonunion and delayed union), accelerated

maturation of newly formed bone and healing time in distraction osteogenesis and reduced time for fracture healing. LIPUS reduced 18–36 days of healing time in conservatively treated fractures, and decreased about 30% of the healing time for surgically managed closed comminuted diaphyseal tibial and femoral fractures (irrespective of implant choice). Open fractures and patients older than 60 years had pronounced benefit from LIPUS treatment. LIPUS' effectiveness increases as soon as treatment is initiated. In addition, fractures of the metatarsal, radius, scaphoid, ankle, fibula and ulna exhibited better healing rates. Smoking, diabetes, vascular insufficiency, osteoporosis, cancer, rheumatoid arthritis and obesity are risk factors for failure. A large cohort of 4190 patients showed 96% healing rate, which is greater than literature averages (93%). In that study, patients between 20 and 29 years old had greater healing rate than patients over 30 years old. Furthermore, LIPUS has no reported adverse effects [37, 56, 86, 125–132].

Only electrohydraulic devices investigated the beneficial effects of ESWT for bone defects and fracture healing. Energy density varied from 0.16 to 0.62 mJ/mm^2, 500–6000 impulses, single or multiple sessions. Increased callus formation; biomechanical properties; ALP activity; and expression of BMPs, IGF-1, eNOS, TGF-β1 and VEGF were reported. Patients subjected to ESWT exhibited better pain scores and decreased nonunion rates, but no difference of fracture-related complications rate. Reported complications include skin petechiae, scarring to the muscle at the treatment site (only for small animals) and subcutaneous swelling. No neuronal damage has been reported even for vertebral exposure (study with small animals) [54, 80, 91, 95, 98, 133–136].

6.2.1. Diabetes

Diabetes is a systemic disease that affects bone healing. Therefore, diabetic individuals are at risk of developing delayed unions, nonunions and pseudarthrosis. Those individuals may also exhibit impaired biomechanical strength of newly formed bone. LIPUS does not increase cellular proliferation during fracture healing in diabetic animals but increases bone healing and biomechanical properties. Additionally, LIPUS increases the expression of TGF-β1 and VEGF but not the expression of IGF-1 and PDGF-β. There are no reports on ESWT and RPWT in diabetic animals or individuals [137, 138].

6.2.2. Osteoporosis

Fracture healing slows and endochondral ossification is impaired with senescence. At the molecular aspect, fracture-induced cox-2 expression in aged rats is lower than youngsters. Thankfully, bone cells keep their mechanosensitivity; as such, acoustic stimulation accelerates fracture healing. It has been shown that LIPUS accelerates fracture healing in estrogen-deficient osteoporotic bone and regains biomechanical strength so that it becomes comparable to non-osteoporotic bones also subjected to LIPUS. Furthermore, LIPUS increases the activity of ALP, and the expression of aggrecan, BMP-2/4/6, cbfa-1, cox-2, FGF-2, OPG, osteocalcin, osterix, RANKL, TGF-α1, VEGF and types I, II and X collagen. The effects of ESWT and RPWT were not investigated for fractures in osteoporotic bones [139–141].

6.2.3. Bone-implant osseointegration

Osseointegration of implants is an important step for recovery of biomechanical strength of bone. Facilitation of this biological process may decrease recovery time and the risk of hardware failure. LIPUS accelerates osseointegration of titanium screws in tibias and femurs, porous hydroxyapatite ceramic and miniscrew implants. Histologically, LIPUS-induced osseointegration provides denser trabecular microstructure at implant-bone interface and thicker newly formed bone. Those findings suggest acoustic therapy may be used as adjunctive therapy to increase hardware lifetime (e.g. for arthroplasties) and decrease recovery time. No reports were found on the subject for ESWT and RPWT [142–144].

6.2.4. Bone graft substitutes

Bone graft substitutes provide an osteoconductive scaffold for filling large osseous defects, and they are an alternative for autologous bone graft, which adds morbidity to the patient. Acoustic therapy provides osteoinductive stimulation for bone. Therefore, combination of acoustic therapy and bone graft substitutes may be a finer alternative to treat fractures associated with large defects. A report showed LIPUS increased bone formation in ulna defect filled with β-tricalcium phosphate (bone graft substitute). In addition, LIPUS did not alter resorption rate of the bone graft substitute. The influence of ESWT and RPWT on large osseous defects filled with bone graft substitutes needs to be explored [86].

6.2.5. Bone-tendon junction

Healing at bone-tendon junction is crucial for tendon repairs (e.g. quadriceps tendon repair, rotator cuff repair, calcaneal tendon repair) and ligament reconstruction (e.g. anterior cruciate ligament of the knee reconstruction and medial patellofemoral ligament reconstruction) to ensure early recovery and improved biomechanical strength. Acoustic therapy may be used as adjunctive therapy in those situations since LIPUS and ESWT were found to enhance healing of bone-tendon junction. Histologically, those acoustic therapies promoted better remodelling of the newly formed trabecular bone, increased bone mineral density and improved tendon-to-bone collagen fibre reconnection [145–147].

6.2.6. Stress fractures

Stress fractures are pathological overuse injuries common in athletes and military recruits. Those injuries result from repetitive loading beyond the regenerative capacity of bone, and represent failure of the adaptive mechanisms of bone to mechanical loads. Results regarding this subject are variable.

LIPUS at 30 mW/cm^2 used to treat incomplete stress injury of the posteromedial tibia, fibula, or second to fourth metatarsals was ineffective to accelerate recovery during a 4-week treatment. On the other hand, LIPUS at 100 mW/cm^2 accelerated stress fracture healing of ulnae even in the presence of non-steroidal anti-inflammatory drugs, which normally delay fracture healing. In addition, athletes with delayed or nonunions of stress fractures of tibia or fifth

metatarsus experienced bone healing within 6–14 weeks of exposure to electromagnetic ESWT [148–150].

6.3. Intact bone

Despite fractures, bone is subject to other diseases that alter its biomechanical strength, such as osteoporosis; or produce disabling pain, such as avascular necrosis of the femoral head. Acoustic therapy may be used for prevention and treatment of some bone disorders.

6.3.1. Healthy bone

It is not known how healthy and intact bone reacts to acoustic loading. Most studies focus on pathological conditions, such as fractures and osteoporosis. The understanding of the normal response of bone to acoustic loads within the physiological range and overuse range is required to ameliorate the comprehension of tissue behaviour in pathological situations, and to prevent some disorders; for instance, stress fractures and osteoporosis.

Intact and healthy bones subjected to LIPUS experience increased density of trabecular spongiosa, and increased activity of FAK, ERK-1/2 and IRS-1. Electrohydraulic ESWT (from 0.15 to 0.47 mJ/mm^2, 500–6000 impulses, single session), in turn, promotes angiogenesis, increased cellular population and bone formation, increased activity of ERK-1/2 and Akt, and increased TGF-β1 production, but no difference on biomechanical tests was found following ESWT exposure [54, 79, 90, 95, 151–153].

6.3.2. Osteoporosis

Studies demonstrated that LIPUS does not increase bone mineral density of osteoporotic bones and does not prevent osteoporosis as measured by dual energy X-ray absorptiometry. However, in those studies the exposure to LIPUS occurred within a short time (from 4 to 12 weeks), and the population of some investigations was heterogeneous. Additionally, histological and molecular analysis of osteoporotic bones subjected to LIPUS showed increased bone formation, normal density of trabecular spongiosa, decreased disruption of trabecular spongiosa and greater expression of cbfa-1 (although lower than controls) [37, 153–157]. Therefore, the authors believe LIPUS possesses beneficial effects for treating osteoporosis.

Electromagnetic ESWT exhibited more pronounced effects on osteoporotic intact bones than LIPUS since ESWT showed increased bone mineral density and decreased bone loss [158].

6.3.3. Immature bone

Concern exists about possible negative effects of ESWT on ephiphyseal plaque in skeletally immature individuals; therefore, ESWT is not formally indicated for children. Contrarily, LIPUS is not contraindicated for skeletally immature individuals. Two studies addressed the effects of ESWT on epiphyseal plaques of animals. It was found that electrohydraulic or electromagnetic ESWT, from 0.38 to 0.60 mJ/mm^2, 1500–3000 impulses, single or multiple sessions, did not harm epiphyseal plaque cells and did not impair growth. Furthermore,

histological analysis revealed increased number of chondrocytes in the proliferative zone and increased thickness of the epiphyseal plaque, suggesting a possible role for growth stimulation. No studies were found for LIPUS that could suggest a possible role for growth stimulation in skeletally immature individuals [96, 97].

6.3.4. Avascular necrosis of the femoral head

Patients who develop avascular necrosis of the femoral head experience groin pain and disability, and further may necessitate joint replacement. A novel possible approach for initial stages of that condition, when bone collapse and osteoarthritis have not established yet, is acoustic therapy. Experimental studies with avascular necrosis of the femoral head models showed that LIPUS and electrohydraulic ESWT increase neovascularization, osteogenesis, osteogenic differentiation of bone marrow cells, decreased size of fat cells—which substitute dead bone—and biomechanical strength of bone. Increased expression of proliferative factors, such as BMP-2, FGF, IGF-1, NO and VEGF, was also found. Furthermore, a clinical and an experimental research revealed that electrohydraulic ESWT may be more effective than core decompression and non-vascularized fibular grafting in patients with early-stage disease; reverts osteonecrosis by one stage; decelerates, or stops, disease' progression; and decreases pain and functional disability [10, 38, 149, 159, 160].

7. Future directions

Undoubtedly, acoustic devices are useful tools to stimulate osteogenesis. Nevertheless, there is a wide list of topics that require further investigations: physical phenomena elicited by acoustic forces need to be proved in vivo, signalling molecules need to be assigned to specific signalling pathways, the control of cellular response to acoustic loads needs to be clarified, RPWT and piezoelectric ESWT influence on bone biology lack investigations, clinical protocols for ESWT and RPWT should be established and, finally, randomized controlled trials addressing acoustic therapy should be performed. As a conclusion, a lot of research is expected within the next years to clarify the unanswered questions about the relationship of bone tissue and acoustic forces.

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