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Review article

External devices increasing bone quality in animals: A systematic review

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ABSTRACT

Background: Osteoporosis can reduce bone quality and increase the risk of fractures. In addition to pharmacological approaches, physical activity, and implanted devices, external devices can also be detected in the literature as a technique to strengthen bones. This type of intervention arises to be particularly promising because it minimizes the invasiveness of therapy. *Methods:* A systematic review of the technologies involved in such devices was carried out to identify the most fruitful ones in improving bone quality. This review, according to the PRISMA Statement, focuses on studies involving animals, and excludes pharmaceutical approaches. *Findings:* The animal models and devices used, their settings, interventions, outcomes measured, and consequent effect on bone quality are reported for each detected technology. Ultrasound and laser arose to be the most studied technologies in the literature, even if they have yet to be proved to have a significant effect on bone quality. *Interpretation:* External devices for bone quality improvement offer a non-invasive approach that causes minimum discomfort to the patient. This review aimed to detect which technologies reported in the literature significantly affect bone quality. The results showed that several technologies are currently used to improve bone quality. However, each study measures different outcomes and uses different measurement methods, device settings, and interventions. This lack of standardization and the reduced number of articles found do not allow for proper quantitative comparisons.

1. Introduction

The skeleton fulfils several fundamental tasks, ranging from enabling motion, protecting vital organs, and providing calcium reserves [1]. Promoting bone health should be paramount for preserving good quality of life and, in particular, in old age when bone health naturally tends to diminish. Ageing leads to an increased exposure to the risk of osteoporotic fragility fracture. In the US only, more than 10 million people aged 50 and over suffer from osteoporosis, and approximately 1.5 million people suffer fragility fractures each year [2–4]. It was estimated that more than 200 million people worldwide had an osteoporotic hip fracture [5]. In

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addition to ageing, also steroid deficiency (e.g., during menopause) can be associated with an increased risk of osteoporosis-related fractures.

Osteoporosis is a skeletal disease characterized by the microarchitectural deterioration of the bone tissue and loss of bone mass, which rapidly degrades bone quality and strength, and increases fragility and the risk of fractures [4–7]. The bone tissue, primarily composed of collagen and mineral, is continuously lost through resorption and rebuilt by formation. Osteoporosis occurs due to an imbalance in the bone turnover in which the resorption rate is higher than the formation one [5,7]. Menopause and advancing age may cause such an imbalance, but several other factors can lead to osteoporotic fractures, e.g., physical inactivity, smoking, alcohol consumption, nutrition, genetic factors, use of glucocorticoids, and endocrine disorders [5,6]. Although the term is widely discussed in clinical debates [8], "bone quality" refers to a concept that involves several characteristics affecting bone strength, among which the extent of mineralization, the microdamage stimulating remodeling, the osteocyte apoptosis rate, changes in the collagenous bone matrix, and bone micro-architecture, composed of trabecular and cortical bone [9,10]. The bone mass condition is typically evaluated using a densitometric method, measuring the Bone Mineral Density (BMD) that is a significant (but not the only) predictor of osteoporosis. As the World Health Organization (WHO) defined, osteoporosis is related to a BMD T-score equal to or less than -2.5, using standard deviation scores of BMD depending on peak bone mass in healthy young women [5–7]. So, higher BMD leads to a lower risk of complications from osteoporosis [6]. Also, such a disease makes difficult any prosthesis implantation. Some modern prostheses are bone anchored by osseointegration, thus avoiding debilitating problems related to soft tissues, providing physiological weight-bearing, improving range of motion, and sensory feedback [11,12]. For these systems, bones play the critical role of anchor site, and implant stability has been found to be correlated to the BMD [13].

Due to the number of people suffering from osteoporosis, several therapies have been proposed to improve bone quality and prevent resorption. A traditional therapy prescribes vitamin D and, eventually, calcium supplements, while different pharmaceutical approaches involve hormonal [14] or other drug treatments [3,7]. Although these therapies look promising [3], they are not the only options available. It was shown that mechanical loading, for example, during weight-bearing exercises, promotes bone formation, especially in the most stressed regions [1,15]. For this reason, physical exercise is prescribed for bone resorption prevention or skeletal improvements [16], usually accompanied by correct eating habits and limited use of alcohol, cigarettes, and coffee [5]. Other modern studies tested some implanted devices for bone healing: in [17], implanted polymeric membrane materials around the fracture can induce effective negative pressure to achieve both membrane- and negative pressure-induced bone regeneration; in [18], a miniaturized electromagnetic device generating Pulsed ElectroMagnetic Fields (PEMFs) therapy was implanted, while [19] investigated the effect of vagus nerve stimulator on BMD in epileptic patients, used for treating refractory seizures in adults and children. However, these methods can involve side effects, such as reliance on long-term patient commitment or invasive surgical procedures, which may be dangerous for other bodily functions [3].

Studies exploiting external devices for bone stimulation with different operating technologies have also been detected in the literature, showing interesting results [15,20–23]. These have the potential to promote bone healing and growth with a non-invasive approach, and minimum discomfort for the patients, avoiding the pharmacological therapy. Some mechanisms through which the strengthening is achieved may be bone mechanical loading, promoting bone growth where the stresses are more significant [1], microfracture provocation and healing [24], metabolism stimulation, stem cell biasing toward bone formation [25], or their combinations. However, to the best of the authors' knowledge, a widely accepted standard methodology for assessing the effectiveness of a technological application on bone quality does not exist.

Given these premises, in this article, a review of the emerging literature that particularly focuses on stimulation from external devices will be conducted to identify whether evidence supports any current technologies as able to improve bone quality. Specifically, bone quality was considered to understand if there are technologies that can influence the bone turnover, and how they operate. Only external devices and the technologies employed, but no other therapies, were considered in this review. They represent an interesting solution for improving bone quality, enabling to avoid invasive approach (e.g., surgical approach for implanted devices), and the pharmacological one, not always usable for the patients involved due to other diseases they suffer from. For a complete overview of the topic, this review is focused on animal studies to lay the groundwork for a subsequent study on humans. Thus, the following research questions will be addressed: "Which external devices or technologies have been tested for bone quality improvement? Which effects do these technologies have on bone quality, and how are measured (outcomes and measurement methods)? Which animal models have been frequently used? Which setting parameters should be used to stimulate bone formation significantly? Which intervention timeline should be used to have appreciable effects on the bones?" The aims of this review are to identify what devices or technologies have been examined, what animal models, stimulus dosage, outcome measures and effects on bone quality have been measured.

2. Methods

2.1. Data source and search terms

This systematic review was conducted by searching articles in four databases (Web Of Science, PubMed, Embase, and IEEXplore) and using the PRISMA Statement checklist [26]. The review was registered in Prospero beforehand (ID number: CRD42022303286). The query string (adapted according to the requirements of each database) used for the research was "((Bone Mass) OR (Bone Turnover) OR (Bone Metabolism) OR (Bone Density) OR (Bone Loss) OR (Bone Losses) OR (Bone Densities) OR (Bone Mineral Density) OR (Bone Mineral Densities) OR (Bone Mineral Content) OR (Bone Mineral Contents) OR (Bone Resorption)) AND ((Biomedical Technologies) OR (Health Technology) OR (Health Care Technology) OR (Supplies) OR (Inventor*) OR (Device*) OR (Equipment)

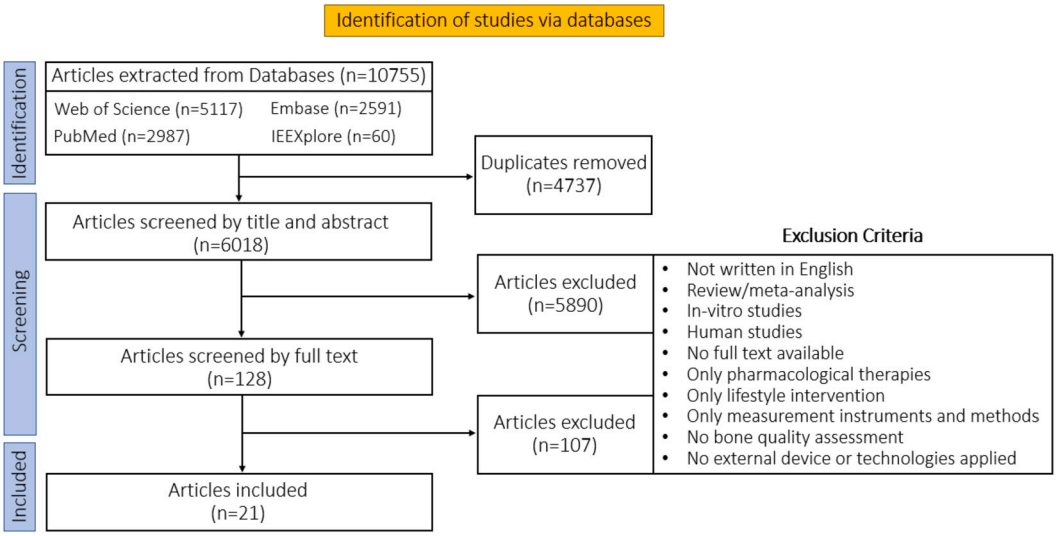

Fig. 1. PRISMA flow diagram for studies selection.

OR (Instrument*) OR (Apparatus) OR (Appliance*)). Terms and keywords were located within the title and/or abstract and/or keywords.

The following criteria were used to define our research: only original full-text articles published in English in the last 20 years about devices that directly improve the bone quality on animals, which were not reviews or meta-analysis, were included in this review.

2.2. Inclusion and exclusion criteria, and study selection

Among those identified from the database search, duplicate articles were excluded using EndNote functionalities and then manually. The remaining articles were screened by reading their title and abstract firstly and then the full text of only the selected papers. Studies were included if they met the following criteria: i) written in English, ii) not reviews or metanalysis, and iii) full text available. Papers were excluded if: i) presented in-vitro studies; ii) presented human studies; iii) employed only pharmacological therapies without the use of external devices; iv) described only lifestyle interventions (physical activity and/or dieting) without the use of external devices; v) described only measurement instruments and methods without the use of external devices; vi) did not provide bone quality assessment; vii) did not apply external devices (not implanted through surgery) or technology. Any studies that propose interventions combined with external devices were included if the devices' effect can be derived.

2.3. Data extraction

The authors (W.S., N.S., C.B., A.I.M., L.B.) extracted the following information from the full-text papers of the eligible studies: the first author and publication year for identification, the animal models, devices used, their settings, the interventions, outcomes measured to evaluate the effect on bone quality, how they are measured, how they change due to the technology application, and if variation is significant according to the statistical analyses. Moreover, data extracted were organized through the physical actions detected: hyperbaric oxygenation, ultraviolet irradiation, mechanical vibrations, magnetic fields, ultrasound, and laser.

2.4. Quality assessment

The quality assessment of the selected articles was performed independently by two authors (A.I.M., G.B.) following the SYRCLE's tool guidelines for assessing the risk of bias [27]. Specifically, this tool covers six types of bias (selection, performance, detection, attrition, reporting bias, and other), and ten domains: sequence generation, baseline characteristics, allocation concealment, random housing, caregivers/investigators blinding, random outcome assessment, outcomes assessor blinding, incomplete outcome data, selective outcome data, and other sources of bias. As reported in Table 1, the authors provided an answer to each question (yes, no, or unclear), a correlated risk of bias (low, high, or unclear), and a motivation for each item and article. Disagreements were solved through consensus-oriented discussion. Furthermore, a third reviewer (L.B.) took the final decision if no consensus was reached.

3. Results

The selection of the included papers was performed as shown in the PRISMA flow diagram in Fig. 1. According to the abovementioned research string, 10755 articles were identified for this systematic review from the four databases queried (Web Of Science,

Table 1
SYRCLE's RoB tool. The type of bias is distinguished as: A) Selection Bias, B) Performance Bias, C) Detection Bias, D) Attrition Bias, E) Reporting Bias, F) Other. The domains are indicated with numbers from 1 to 10: 1) Sequence Generation, 2) Baseline Characteristics, 3) Allocation Concealment, 4) Random Housing, 5) Caregivers/investigators Blinding, 6) Random Outcome Assessment, 7) Outcomes Assessor Blinding, 8) Incomplete Outcome Data, 9) Selective Outcome Data, 10) Other Bias Sources. The correlated risk of bias can be high (black colored) or low (grey colored); the cell is empty (white colored) if the risk is unclear.

Risk of Bias in animal studies according to SYRCLE's Risk of Bias tool

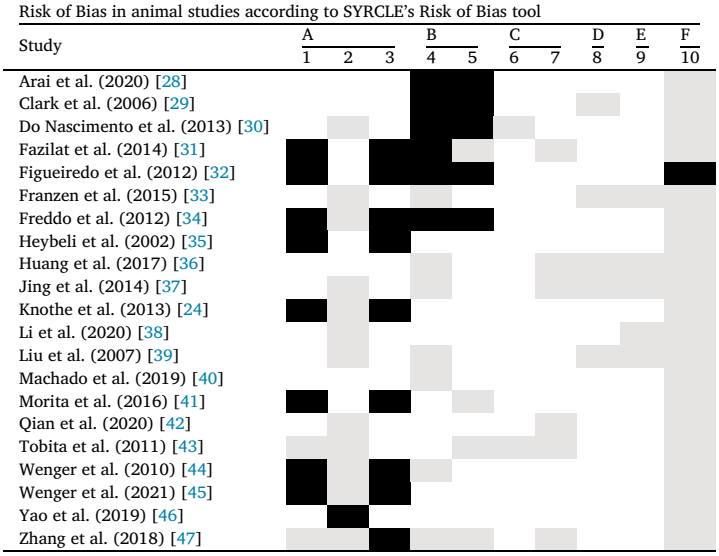

Table 2
The number of papers involved in each technology found in the reviewed literature and those reporting increased, mixed, or not significant effect on bone quality, divided on the basis of the applied technology. (*) highlights that in the resulting number [30] is double-counted due to comparing the effects of applying both laser and ultrasound technologies.

Technology/Effect	Total	Increased	Mixed	Not significant
Ultrasound	6	3	1	2
Laser	6	2	3	1
Magnetic Fields	4	2	0	2
Hyperbaric Oxygenation	1	1	0	0
Mechanical Vibrations	4	2	1	1
Ultraviolet Irradiation	1	0	0	1
Total	22*	10*	5	7

PubMed, Embase, and IEEEXplore). 6018 papers were included after removing duplicates (n = 4737) using Endnote, and manually. Subsequently, 5890 articles were excluded after screening by reading their title and abstract, while 107 articles were excluded after reading the full text of the remaining ones. Finally, 21 papers were included to extract information about the effect of stimulation from external devices on bone quality. Table 2 shows how the included papers are divided into the detected technologies and effect on bone quality resulted. Table 3 and the following sections, which are distinguished depending on the tested technology, summarize for each paper the trial type, animal model and size of the sample, the device used, its setting parameters, the intervention (including how long a session lasts and how many times it is repeated), and measured outcomes, how they are measured and how their change due to the technology application (positive, negative or not notable) affected bone quality, classifying the effect on it. In addition, statistically significant outcomes ($p\text{-value} < 0.05$ or $p\text{-value} < 0.001$, depending on what is declared in the paper) are highlighted.

Six different technologies tested for bone improvement were identified in the literature: ultrasound (six papers out of 21), laser (six), magnetic fields (four), mechanical vibrations (four), hyperbaric oxygenation (one), and ultraviolet irradiation (one). Nine papers out of the total 21 show technologies resulting in an increased effect on bone quality compared to untreated control groups [29,30,35,38,39,42–44,46]. Seven papers report no significant changes to a control group [28,33,37,40,41,45,47]. The five remaining papers [24,31,32,34,36] showed mixed results, meaning that improvement is conditional on some extra constraints, e.g., the reported improvement was limited to a specific time frame, the technology was applied in conjunction with other devices, or neither an improvement nor a worsening of bone quality was recorded in the treated group compared to the worsening of the control group. It is worth noting that [30] proposes a comparison between devices exploiting two different technologies, both resulting in an increased effect on bone quality, as reported in Table 3.

All the research studies proposed clinical trials as clinical outcomes are evaluated. Among the articles giving details of the sample grouping, some papers specified criteria to be followed (Controlled clinical trial in Table 3), while others grouped the sample randomly (Randomized clinical trial). If no details are given, the generic clinical trial is considered.

The most used animal models are rats and rabbits. Custom and commercial devices are used in the included papers, and their settings and intervention time are reported specifically in Table 3.

The outcomes mainly employed for bone quality evaluation are BMD (12 papers), followed by bone and tissue volume rate — BV/TV (ten papers), trabecular parameters (seven papers) — the thickness (Tb.Th), number (Tb.N), and separation (Tb.Sp), and bone volume — BV (five papers). Then, bone mineral content (BMC), bone-specific surface (BS/BV), bone formation (BF), due to bone turnover, and cortical parameters — area (Ct.Ar) and thickness (Ct.Th) — are used (three papers). Finally, volumetric BMD (vBMD), osteoclast number per bone surface (N.Oc/BS), and mineral apposition rate (MAR) are evaluated (two papers). All the outcomes listed in the matching column in Table 3 not mentioned above were detected in only one paper among those included in the review. Such parameters are defined below: bone mass (BM), marrow area (Ma.Ar), percent cortical area (Ct.Ar/Tt.Ar), tissue volume (TV), mineral density in tissue volume (TVD), fractal dimension (FD) that measures the complexity of the morphological bone pattern, high bone density (Hi.D), bone surface per tissue volume (BS/TV), eroded surface (ES), and ES per bone surface (ES/BS), bone resorption (BRs.R), and formation rate per bone surface (BFR/BS), adjusted apposition rate (Aj.AR), osteoid maturation time (Omt), bone mineralization lag time (Mlt), osteoblast number (N.Ob) — per tissue volume (N.Ob/TV) and per bone surface (N.Ob/BS), osteoclast number (N.Oc) — per tissue volume (N.Oc/TV), and bone (N.Oc/BS) and eroded (N.Oc/ES) surface, osteoclast surface per ES (Oc.S/ES), quiescent surface (QS), label surface area (LS), single-labeled surface (sLS), labeled surface per osteoid surface (LS/OS) and bone surface (LS/BS), mononuclear osteoclast number (N.Mo.Oc) — per bone (N.Mo.Oc/BS) and eroded (N.Mo.Oc/ES) surface, and per tissue volume (N.Mo.Oc/TV), mononuclear osteoclast number (N.Mu.Oc) — per eroded surface (N.Mu.Oc/ES) and tissue volume (N.Mu.Oc/TV), structure model index (SMI), tissue mineral density (TMD) and content (TMC), bone volume fraction (BVF), inner (In.Pr) and outer (Out.Pr) perimeter for cortical bone.

These are measured primarily through CT [29,34], μCT [41,36,44,45,37,38,42,47,28,40,43,39], CBCT [30], and DEXA [36,42,35,40,33], combined in some studies with histological and histomorphometric analysis [24,29,31,37,46].

3.1. Hyperbaric oxygenation

In [29], the research team tested HyperBaric Oxygenation (HBO) to increase bone density. Rabbits were treated with 2.4 atm HBO 90 min per day for 20 sessions before and ten after surgery. No limb-device coupling was described, and specific section stimulation was not performed. No further detail is provided on the device since therapy was performed in a military medical center.

HBO was reported to increase bone quality in the first 20 sessions before distraction, having measured through Computed Tomography (CT) and histomorphometric analysis an increase in BMD and bone fill following the treatment (Table 3). The authors suggested to pay particular attention to CT measurements especially after surgery as they can be affected by scattering artifacts. An analysis of variance (ANOVA) was used to evaluate the changes in bone density, highlighting a statistically significant result (p-value < 0.001).

3.2. Ultraviolet irradiation

In [41], the effect of UltraViolet (UV) irradiation (0.54 mW/cm^2 of irradiance) was tested on different groups of mice, distinguished according to the wavelength of the applied irradiation (268, 282, 290, 305, and 316 nm). The treatment session lasted 185 s and had been repeated twice weekly for four weeks. The main trabecular and cortical bone volume parameters measured through μCT tended to be higher for the 316 nm group. Nevertheless, no statistically significant bone quality variation could be identified (Table 3).

3.3. Mechanical vibrations

All the devices exploiting vibration stimulation employed custom-made devices applied only to rats and mice. Stimulus parameters differed in displacement amplitudes, ranging from 1 to 5 mm, accelerations from 0.3 to 1.5 g, and frequencies from 30 to 40 Hz. Additionally, in [46] a 50-mm, 2.5-Hz rotational shaking is applied. Intervention times lasted 21 [36,45] or 60 days [44,46] with a single treatment of 20 min/day [45] and 30 min/day for five or six days per week in [44], [46], respectively. [36] instead proposed sessions lasting 200 s, to be repeated twice daily for 21 days. No device-limb strict coupling was performed. The stimulus was delivered through the cage's platforms as Whole-Body Vibration (WBV). The only exception was [36], where rats' feet were fixed to the footplates with medical tape. Specific devices, settings, interventions, measurement methods, and outcomes are reported in Table 3.

[44,46] claim an increasing effect on bone quality since, globally, vibrations positively affected the outcomes measured (Table 3) compared to the non-stimulated control group. It is worth highlighting that in [44], the different acceleration values tested produced in the femur varied effects regionally, still always significant on bone quality according to the statistical *t-test*. Specifically, a 0.5-g-vibration level (LOW in Table 3) was reported to be more effective for increasing bone density than 1.5 g (HIGH). In [46] instead, significant effects on bone quality were achieved due to the mechanical stimulation both in ovariectomy-induced (OVX) mice, simulating post-menopausal conditions and in wild-type (WT) mice that did not undergo ovariectomy, thus simulating pre-menopausal condition. Besides, [45] showed no significant change by observing the trend of BV and BMD, while [36] reported

Table 3
Results extracted from the included papers: technologies (Tech), trial type, animal and sample size (Sample), devices, settings, interventions, measurement methods, outcomes, and effects on bone quality for each reference (Ref). Hyperbaric Oxygenation, Ultraviolet irradiation, mechanical vibrations, magnetic fields, ultrasound, and laser will be reported as HBO, UV, MV, MFs, U, L, respectively. Positive, not notable, or negative effects on the listed outcomes are reported with (+), (NN), and (-), respectively. The superscript * means that the measured outcome variation has a *p-value* < 0.05, while ** implies a *p-value* < 0.001.

Ref	Tech	Trial type	Sample	Device	Settings	Interventions	Measurement methods	Outcomes	Effect on bone quality
[29]	HBO	Randomized clinical trial	Rabbits (n = 20)	Provided by the Hyperbaric Oxygen Service, Eisenhower Army Medical Center	Pressure: 2.4 atm	90 min per day, 20 sessions before and 10 after surgery	CT and histo-morphology	(+) on **BMD**** and **Bone fill***	Increased
[41]	UV	Clinical trial	Mice (n = 42)	UV lamps of the LED system from Nikkiso Co ltd (Japan)	Wavelength: 268, 282, 290, 305, and 316 nm; Irradiance: 0.54 mW/cm^2; Irradiation dose: 1 kJ/m^2	185 s, 2 days per week, 4 weeks	μCT	316-nm wavelength: (+) on **Ma.Ar**, **BV/TV**, **Tb.Th**, **Ct.Ar**; (NN) on **Tb.N**, **Tb.Sp**, **BMD**; (-) **Ct.Th**, **Ct.Ar/Tt.Ar***. Other wavelengths: (+) **Tb.N**, **Tb.Sp**, **Ma.Ar**; (NN) on **BV/TV**, **Tb.Th**; (-) **BMD**, **Ct.Ar***, **Ct.Ar/Tt.Ar***, **Ct.Th***	Not significant
[46]	MV	Controlled clinical trial	Mice (n = 24)	Custom comprising: a shaking device (NX-25D) and a vibration device (SK-40-D1) by Nissin Scientific Corporation (Japan)	Frequency: 40 Hz, Amplitude: 5 mm, Shaking: 2.5 Hz, 50 mm	30 min per day, 6 days per week, 10 weeks	Histomor-phometry	Stimulated OVX to OVX: (+) **BS/TV***, **Tb.N***, **Omt***, **N.Ob***, **Mlt***, **N.Oc/ES***, **Oc.S/ES***, **N.Ob/TV***, **sLS***, **N.Mu.Oc/ES***, **N.Mo.Oc/ES***; (-) **BS/BV***, **Tb.Sp***, **MAR***, **Aj.Ar***, **ES/BS***, **ES***, **BRs.R***. stimulated WT to WT: (+) **Omt***, **BV***, **Tb.N***, **BS/TV***, **LS/OS***, (-) **ES/BS***, **MAR***, **Aj.Ar***, **BS/BV***, **ES***, **N.Mu.Oc/TV***. Stimulated OVX to stimulated WT: (+) **Aj.Ar***, **N.Mo.Oc***, **N.Mo.Oc/BS***, **N.Oc/ES***, **N.Mo.Oc/TV***, **N.Oc/TV***, **N.Mu.Oc***, **N.Mu.Oc/ES***, **N.Mo.Oc/ES***, **N.Oc/BS***, **N.Mu.Oc/TV***, **OV/BV***, **MS/BS***; (-) **QS***, **BV***, **BV/TV***, **LS/OS***, **BRs.R***, **Tb.Sp***. Stimulated (no OVX) to not stimulated: (+) **N.Oc/TV***, **LS/BS***, **N.Oc/BS***, **MS/BS***; (-)**QS***, **BV***, **BV/TV***, **LS***.	Increased
[36]	MV	Randomized clinical trial	Rats (n = 40)	Custom device for passive exercise and local vibration on hindlimbs	Frequency: 35 Hz, Amplitude: 1 mm	200 s, 2 times per day, 21 days, 20 bouts in 2 s each time with 8s intervals	DEXA, μCT	TS: (+) **BS/BV***, **Tb.Sp*** (NN) cortical **vBMD**, (-) trabecular **vBMD***, **BV/TV***, **Tb.Th***, **Tb.N***; TSP: (+) **BS/BV***, **Tb.Sp*** to TS, TSPV (NN) cortical **vBMD**, (-) trabecular **vBMD***, **BV/TV*** to TSPV, **Tb.Th*** to TSPV, **Tb.N** to TS, TSPV; TSV: (+) **BS/BV***, (NN) cortical **vBMD**, (-) **BV/TV*** to the other groups, **Tb.Th**, **Tb.N*** to TS, **Tb.Sp*** to TS; TSPV: (+) trabecular **vBMD*** to the other groups, **BV/TV** to TS, **Tb.Th*** to TS, (NN) trabecular **vBMD**, cortical **vBMD**, (-) **BS/BV***, **Tb.N*** to TS, **Tb.Sp*** to TS.	Mixed
[45]	MV	Controlled clinical trial	Mice (n = 48)	Custom device powered by a servomotor (Mavilor BLT-055, Spain) connected to a polycarbonate platform	Frequency: 30 Hz, Acceleration: 0.3 g	20 min per day, 21 days	μCT	(NN) on **BV**, **TV**, **BV/TV**, **BMD**; (-) **TVD***	Not significant

Table 3 (*continued*)

Ref	Tech	Trial type	Sample	Device	Settings	Interventions	Measurement methods	Outcomes	Effect on bone quality
[44]	MV	Controlled clinical trial	Mice (n = 36)	Custom vibration platform	Frequency: 32 Hz, Acceleration: 0.5 (LOW) or 1.5 (HIGH) g	30 min per day, 5 days per week, 3 months	μCT	Femoral head: (i) LOW, (+) **BV**, **BV/TV**, **Tb.Sp**, **FD***, **Hi.D***, (NN) **Tb.Th**, (-) **Tb.N**; (ii) HIGH, (+) **BV/TV**, **Tb.N**, **Hi.D***, (NN) **BV**, **Tb.Th**, **FD**, (-) **Tb.Sp**; Femoral neck: (i) LOW, (+) **BV**, **FD**, **Hi.D*** (NN) **BV/TV**; (ii) HIGH, (+) **Hi.D***, (NN) **BV**, **FD**, (-) **BV/TV**; Diaphysis: (i) LOW, (+) **FD***, **Hi.D***, (NN) **BV**, **BV/TV**; (ii) HIGH, (+) **FD**, **Hi.D**, (NN) **BV/TV**, (-) **BV**; Condyle: (i) LOW, (+) **Tb.Sp**, **FD**, **Hi.D**, (NN) **BV/TV**, **Tb.Th**, (-) **BV**, **Tb.N**; (ii) HIGH, (+) **BV**, **Tb.Sp**, **FD***, **Hi.D**, (NN) **BV/TV**, **Tb.Th**, **Tb.N**.	Increased
[37]	MFs	Randomized clinical trial	Rats (n = 32)	CRSMART-C, Chaoruishi Medical Supplies Co. (China)	Rotating MF, MF_{max}: 400 mT, MF distribution: 0.38-0.60 T, Frequency: 7 Hz	2 h per day, 4 weeks	μCT, histology, histomorphometry	HU to control group: (+) **Tb.Sp***, **N.Oc/BS**, **SMI***, (-) **BMD***, **Tb.N***, **Tb.Th***, **BV/TV***, **Ct.Th***, **Ct.Ar***, **N.Ob/BS**, **MAR***, **BFR/BS***. HU+RMF to HU group: (NN) **Tb.N**, **Tb.Th**, **Tb.Sp**, **Ct.Ar**, **Ct.Th**, **N.Ob/BS**, **N.Oc/BS**, **BMD**, **BV/TV**.	Not significant
[38]	MFs	Randomized clinical trial	Rats (n = 56)	PS30, Orthofix Medical Inc. (USA)	PEMF, Frequency: 15 Hz, MF: 30 T/s	3 h per day from day 7 to 1, 3 or 4 weeks after distraction	μCT	At 3 weeks after distraction: (NN) **BMD**, **BV/TV**; at 4 weeks after distraction: (+) **BMD***, **BV/TV***	Increased
[42]	MFs	Randomized clinical trial	Rabbits (n = 40)	TY-PEMF-B, Tianjin Tongye Technology (China)	PEMF, Frequency: 15 Hz, MF: 3.8 mT	40 min per day, 5 days per week, 8 weeks	DEXA, μCT	PEMF: (+) **BMD***, **BV/TV***, **Tb.N***, **Tb.Th***, (-) **Tb.Sp***; Scl-Ab: (+) **BMD***, **BV/TV***, **Tb.N***, **Tb.Th***, (-) **Tb.Sp***; PEMF+Scl-Ab: (+) **BMD***, **BV/TV***, **Tb.N***, **Tb.Th***, (-) **Tb.Sp***.	Increased
[47]	MFs	Randomized clinical trial	Mice (n = 19)	Custom 16 T superconductive magnet (Japan)	Static MF, MF: 0.2 T	Continuously exposed for 30 days	μCT	(NN) trabecular **BMD**, **BMC**, **TMD**, **TMC**, **BV**, **BV/TV**, **BVF**, **BS/BV**, **Tb.Th**, **Tb.N**, **Tb.Sp**, cortical **BMD**, **BM**, **Ct.Ar**, **In.Pr**, **Out.Pr**, **Ct.Th** in both HyMF and MMF groups to the control	Not significant
[28]	U	Randomized clinical trial	Rats (n = 26)	Osteotron D2 ITO (Japan)	Frequency: 1.5 MHz, Intensity: 30 mW/cm^2, Pulse repetition frequency: 1 kHz	20 min per day, 14 days	μCT	(+) Alveolar **BF***, **intermolar width***, **N.Oc***, (NN) **BMC**, **BV/TV**	Not significant
[30]	U	Clinical trial	Rabbits (n = 24)	Sonopulse III Indústria Brasileira de Equipamentos Médicos (Brazil)	Frequency: 1 MHz, Intensity: 2 W/cm^2	6 min per day, 24 days	CBCT	(+) to the control and laser groups **BMD*** recuperation	Increased
[35]	U	Clinical trial	Rats (n = 32)	Diagnostic sonographic equipment - AU3 Partner, Esaote (Italy)	Frequency: 7.5 MHz, Intensity: 11.8 mW/cm^2, Pulse duration and repetition frequency: 1 ms, 1 Hz	10 min every 5 days, 1 (C), 5 (B), 8 (A) times	DEXA	Fracture region and whole bone: (i) A, (+) **BMD***, (ii) B, (+) **BMD***, (iii) C, (+)**BMD**	Increased
[24]	U	Clinical trial	Rats (n = 20)	Modulith SLX lithotripter Storz Medical AG (Switzerland)	Frequency: 2 GHz, Energy density: 0.46, 1.06 mJ/mm^2, Shock waves: 500, 1500	One-time treatment	Histomorphometry	Normal activity+microdamage: (i) 1500, (+) **BF*** at both power levels, (i) 500, (NN) **BF**. Suspension: (+) **BF*** in treated to baseline and contralateral groups and in contralateral to suspended or baseline controls, **bone resorption*** to contralateral, suspension and baseline controls; (NN) **BF** in suspended to baseline controls	Mixed

(*continued on next page*)

Table 3 (*continued*)

Ref	Tech	Trial type	Sample	Device	Settings	Interventions	Measurement methods	Outcomes	Effect on bone quality
[40]	U	Clinical trial	Rabbits (n = 17)	EXOGEN Bioventus (USA)	Frequency: 1.5 MHz, Intensity: 30 mW/cm^2, Pulse duration and repetition frequency: 200 ms, 1 kHz	20 min per day, 18 days	μCT, DEXA	(NN) **BMD**, **BMC**	Not significant
[43]	U	Controlled clinical trial	Rabbits (n = 42)	SAFHS 2000J Teijin Pharma (Japan)	Frequency: 1.5 MHz, Intensity: 30 mW/cm^2, Pulse duration and repetition frequency: 200 μs, 1 kHz	20 min, 6 times per week, 4 (A), 6, 8 (B) weeks	X-ray, μCT	B to A: (+) **vBMD**** in total, external, internal, **vBMD***, **BV*** in endosteal zone. B to control: (+) **vBMD*** external and internal zones, **BV**** in internal zone. (NN) for other comparisons	Increased
[30]	L	Clinical trial	Rabbits (n = 24)	Flash Lase III, DMC, Sao Carlos (Brazil)	Wavelength: 808 nm, Output power: 100 mW, Energy density: 6 J/cm^2	6 min per day, 24 days	CBCT	(+) to the control and ultrasound groups **BMD*** recuperation	Increased
[31]	L	Clinical trial	Rabbits (n = 18)	THOR Photomedicine Ltd	GaAlAs Wavelength: 810 nm, Power: 200 mW, irradiation mode, continuous wave, Energy and power density: 3 J/cm^2, 400 mW/cm^2	7.5 s every other day, 14 days	Histology	Days after distraction: (i) 10, (+) **BF*** to control; (ii) 20, (+) **BF*** to control, (-)**BF** to (i); (iii) 40, (+) **BF** to control, (-) **BF** to (i), (ii)	Mixed
[32]	L	Clinical trial	Rats (n = 20)	Low Level diode laser - MMOptics, São Carlos (Brazil)	Wavelength: 780 nm, continuous, Power: 40 mW, Energy density: 10 J/cm^2	10 s on alternate days, 15 days	Weibel procedure	LLLT: (+) **BM**; MPA: (+) **BM**; LLLT+MPA: (+) **BM***	Mixed
[33]	L	Randomized clinical trial	Rats (n = 61)	GaAlAs diode laser device - Rønvig Dental AS, Daugaard (Denmark)	GaAlAs Continuous wavelength: 830 nm, Power: 75 mW, Power and energy density: 550 mW/cm^2, 23 J/cm^2, 3 J per session	17 s, every 2nd/3rd day for 1, 3, 5, 7, 14, 21 days receiving 1, 2, 3, 4, 5, 7 doses of irradiation, respectively	X-ray	(NN) **BMD**	Not significant
[34]	L	Clinical trial	Sheep (n = 5)	Thera Laser - DMC, São Carlos (Brazil)	GaAlAs Wavelength: 830 nm, Energy density: 5 J/cm^2 at 3 points (15 J/cm2 total), Power: 50 mW, continuous wave	1.41 min immediately after the last activation and then every 48 h, for 8 sessions	CT	Latency/activation period: (-) **BF**; consolidation period: (+) **BF**	Mixed
[39]	L	Randomized trial, double-blind study	Rabbits (n = 15)	Low-energy laser apparatus - Phototherapy, San Diego (USA)	Wavelength: 830 nm, Energy and power density: 10 J/cm^2, 200 mW/cm2	50 s at 4 points, daily, 20 days	μCT	(+) **BMD**** in fractures	Increased

a conditional effect over two different control groups: one consisted of rats suspended by their tails to generate osteoporosis and simulate space conditions by reducing loading on weight-bearing bones, while in the other, rats were not suspended. The sampling 40 rats were divided into five equal groups: tail-suspension (TS), TS with 35-Hz vibration (TSV), TS and passive exercise (TSP), TS and passive exercise with 35-Hz vibration (TSPV), and control. BMD, measured through dual-energy X-ray absorptiometry (DEXA) and microcomputed tomography (μCT), decreased in the TS and TSP groups compared to the control, but this one had no statistically significant variation with TSV and TSPV. TSPV group showed a significant increase in BMD to TS but not to TSV group. Thus, the paper was considered among those with mixed results.

3.4. Magnetic fields

Three different animal models were used in the included papers: rats [37,38], mice [47], and rabbits [42].

Three subgroups were identified among the Magnetic Fields (MFs) employed: in [37], a rotating MF with 0.38-0.60 T flux and a 7-Hz magnet rotation creating a non-uniform field was applied through a commercial device; commercial devices were also used in [38,42] to apply PEMFs with 30 T/s and 3.8 mT, respectively, both at 15 Hz of frequency. Finally, in [47], a custom 16 T superconductive magnet was used to enforce an in-cage static MF of 0.2 T. Intervention times lasted two h/day for four weeks [37], three h/day from day seven after surgery up to four weeks [38], 40 min/day for five days/week for eight weeks [42]. In contrast, [47] proposed a continuous exposition for 30 days. Specific devices, settings, interventions, outcomes, and measurement methods are detectable in Table 3.

As well as vibrating devices, MFs are reported to have diverse effects on bone quality. Significant positive effects were measured on BMD and BV/TV in [38] and also trabecular bone parameters in [42] after applying MF according to the settings and interventions reported in Table 3, thus determining an increased effect on bone quality. It is worth noting that in [38], the MF significative positive effect was observable in the group terminated after four weeks, while in [42], the BMD measured in the group of OVX rabbits treated with PEMF and Scl-Ab injection was significantly higher than that measured in groups of OVX rabbits only subjected to PEMF or Scl-Ab injections or untreated. Also, BV/TV, and trabecular parameters in all the treated groups were significantly higher than the control. In contrast, in [37], no statistically significant difference was detected in comparison with a control group in hindlimb unloading conditions through rats' tail suspension. Similar results were achieved in [47] by comparing the outcomes, reported in Table 3, measured in the group treated with a moderate static magnetic field (MMF) to those reached in a control and a 500-nT hypomagnetic field (HyMF) group. The papers reporting an increased effect employed PEMF, while the others used rotating or static fields.

3.5. Ultrasound

According to the article screening, ultrasound is one of the most adopted technologies due to six papers included. The experiments were applied on rats [24,28,35] and rabbits [30,43] by using commercial equipment (Table 3). Therapies were performed with sinusoidal waves ranging from 1 to 7.5 MHz, with intensity between 11.8 mW/cm^2 and 2 W/cm^2, delivered in bursts at a pulse repetition frequency of 1 kHz [28,40,43], with different pulse duration (200 ms in [40], 200 μsec in [43]) or with a 1 ms pulse at 1 Hz [35]. The only exception is for [24], in which 500 or 1500 shock waves at 2 GHz and different power levels were used to bring controlled microdamage on rats' bones and promote appositional growth. Intervention times lasted one-time treatment [24], 20 min/day for 14 days [28] and 18 days [40], 6 min/day for 24 days [30], 10 min/day for one, five, and eight times every five days [35], and 20 min for six days/week for four, six, and eight weeks [43]. Devices, settings, interventions, and outcomes are specified in Table 3.

Among the screened articles, [30,35,43] reported a significant positive effect in BMD, measured through different X-ray and CT instrumentation. In [24], bone apposition measured through histomorphometric analysis was significantly higher in bones treated with 1500 shock waves compared to the contralateral control bones, while no significant increase in new bone formation was observed in the group treated with 500 shocks by exploiting the same power levels. Thus, the paper was assigned to the mixed group due to the conditional results. Finally, [28,40] found no statistically significant variation in Bone Mineral Content (BMC) or BMD values over the control group, thus determining no significant effect on bone quality.

3.6. Laser

Laser is the second most studied technology, with as many papers included as ultrasound. Three different animal models have been used in included papers: rabbits [30,31,39], mice [32,33], and sheep [34]. All authors used commercial devices operating in the infrared spectrum (700 nm – 1 mm), from 780 nm to 830 nm. Output power ranged from 40 to 200 mW. Intervention times lasted 6 min/day for 24 days [30], 7.5 sec/day for 14 days [31], 50 sec/day for 20 days [39], 10 sec/day for 15 days [32], 17 sec for one, two, three, four, five, seven times treatment [33], 1.41 min for eight sessions [34]. Specifically, devices, settings, interventions, outcomes, and measurement methods are shown in Table 3.

The papers reported overall varied results on bone quality improvements. [30,39] showed an increased effect on bone quality after laser therapy application due to the significant increase in BMD measured through Cone-Beam CT (CBCT) and μCT, respectively. A non-statistically significant difference emerged in [33] between BMD values measured through X-ray in the group undergoing Low-Level Laser Therapy (LLLT) and that measured in the control group. The results of the remaining three articles [31,32,34] were considered mixed since the laser effects on bone quality do not depend only on the technology application. [32] reported a

significant increase in bone mass if LLLT was combined with applying a mandibular propulsive appliance (MPA) to rats and no significant variation if only LLLT treatment was applied. [34] reported positive effects on bone formation only if the laser therapy was applied during the consolidation period (the last period of the distraction osteogenesis protocol, preceded by the latency and activation ones). Such an outcome instead tended to decrease if the laser technology was applied during the latency/activation period regardless of the distraction device usage period, with a consequent delay in bone healing. Finally, an increase in bone formation was visible in [31] through histological analysis only in the early stages of the treatment. At the same time, an inflammation response was active.

4. Discussion

Based on this review's database analysis, no prevailing technology is employed for bone quality enhancement in the literature. Besides, animal models, devices, setting parameters, interventions timeline, and their effect on bone quality differed widely from one study to another.

Technologies HBO, UV, mechanical vibrations, MFs, ultrasound, and laser are the technologies detected from this literature review. Specifically, laser and ultrasound resulted the most studied technologies (six papers each out of the 21 included), followed by mechanical vibrations and MFs, and finally by HBO and UV. Beneficial effects on bone quality are mainly reported in ultrasound applications, but the technology that most affect bone quality cannot be deduced due to the few articles that met the research criteria.

Animal models Among those involved, sheep have bone mineral composition, architecture, and remodeling capacity that are the most similar to humans [48]. Nevertheless, this review found only one article that used sheep [34], while all the other articles used small animal models (mice, rats, and rabbits), due to their accessibility, low cost, and ethical acceptance [49]. Due to the reduced number of studies involving animals with human-like bone structure (e.g., sheep), data extracted from the papers included in this review may have a limited impact on human studies.

Devices They depend on the technologies tested in each research paper. Both custom and commercial devices are used in testing all the technologies that arose from the research. It is worth noting that only custom devices are found to be used in case of mechanical vibrations due to their easier feasibility.

Setting parameters and interventions The devices setting parameters are also strictly dependent on the tested technology: pressure was set only in the case of HBO [29], while acceleration and oscillation amplitude were specified for testing mechanical vibrations. The studies in [44,45] employed similar devices with similar vibrating frequencies (30-32 Hz) and accelerations (0.3, 0.5, and 1.5 g). [36,46] defined the vibration amplitude rather than the acceleration, but stimulations were applied at similar frequencies to [44,45] (at 35 and 40 Hz, respectively). Concerning the interventions, it is worth noting that, except for [36] in which 200-s sessions were proposed, in [44–46] mechanical vibrations in sessions lasting 20 o 30 minutes were applied. In all four studies, vibrations at 30-40 Hz were applied for 30 min or less (with a mean time of intervention of 40.5 days), which comply with the 30-50 Hz for less than 30 min recommended in [20] for preventing harmful and promoting good WBV on human bodies, meaning that WBV has a positive effect on bone density, as well as on muscle strength.

Lower frequencies (7-15 Hz) were set for applying MFs [37,38,42], except for [17], in which static MFs were used. Also, it differed in the intervention since the treated sample was continuously exposed to MFs (0.2 T) for 30 days. It is worth noting that among the other research studies, a stronger MF (0.38 - 30 T) was applied in case of longer (2-3 hours) and repeated sessions for less time (four weeks) [37,38], while the opposite (3.8 mT) happened with applying shorter (40 min) and more repeated sessions (eight weeks) [42].

Higher frequencies than MFs and mechanical vibrations, ranging from 1 MHz to 7.5 MHz, were involved in ultrasound tests [28,30,35,40,43]. The duration of each session (20, 10, and 6 min) decreased with increasing ultrasound intensity (30, 11.8, and 2000 W/cm^2), regardless of the whole time of interventions; in particular, [28,40,43] proposed ultrasound application at the same frequency (1.5 MHz), intensity (30 mW/cm^2), pulse repetition frequency (1 kHz), and each session duration (20 min), but differed in the intervention, reaching a mean value of 26 days among these. The exception was [24], in which 500 or 1500 shock waves at two different power levels and a 2-GHz frequency were applied in a one-time treatment.

Laser technology was applied by setting a continuous wavelength in all six included papers, with a common value of 830 nm in [33,34,39], but with different power densities (ranged from 200 to 500 mW/cm^2) and energy densities (ranged from 10 to 23 J/cm^2). Lower but still similar wavelengths (780, 808, and 810 nm) were applied in [30–32] with similar energy density (10 and 6 J/cm^2, respectively). Each session had a mean duration of 90 s, which is less than the 15 min for the ultrasound sessions (except for [24] due to its one-time treatment), 113 min for the MFs ones (except for [47]), or the 1250 s for the mechanical vibration sessions.

A comparable session duration of 185 s, but lower tested wavelengths (268, 282, 290, 305, 316 nm) were detected instead in [41], which is the only paper concerning UV.

Measurement methods and outcomes CT, μCT, and CBCT are the most used measurement methods among those detected in the included papers, and they are also the most predictive for evaluating bone quality, by using the Hounsfield units [50]. Concerning the outcomes, BMD resulted in the most measured parameter, even if it cannot be considered the most predictive parameter of bone quality due to the low number of articles included in this review, and, to the best of authors' knowledge, there is no evidence about this point in the literature [8].

Effect on bone quality No conclusions can be drawn for HBO since, despite showing increased effects on bone quality, it was detected in only one paper [29] for which a medium risk of bias was assessed.

UV irradiation was found to have no significant effects on bone quality in the only one included paper [41] with a medium risk of bias. The research highlighted positive but not significant effects on the outcomes measured in the mice group subjected to UV irradiation with a 316-nm wavelength rather than with the other wavelengths tested or referring to the outcomes measured in the control group.

Mechanical vibrations positively affected the outcomes measured in [44,46]. Different outcomes and also different measurement methods were used. Nevertheless, the outcomes were positively affected at the end of the similar treatments applied, as reported in Table 3 in the Interventions column. Vibrations were still applied in a slightly shorter session for fewer days in [45] resulting in no changes in the treated group. Completely different duration of sessions was considered in [36] but repeated for the same time of intervention employed in [45]. In this case, the group of stimulated and suspended rats was not subjected to BMD-decreasing as it happened, in contrast, to the group of only suspended rats. Consequently, no trend in the device settings and interventions can be identified since [44,36] with a low risk of bias showed an increase and a non-reduction in bone quality to the control group, respectively. Those with a medium risk of bias [45,46] also resulted in different effects, reporting in bone quality no notable difference, the former, and an increase, the latter, to the control. Such a conclusion follows the literature, in which an interesting review about the effect of WBV exercise in postmenopausal osteoporosis women [51] stated that, although WBV is only a recommended physical activity to treat postmenopausal osteoporosis and cannot replace pharmacological and dietary treatments, the relationship between biochemical factors, bone structure, and vibration setting parameters in this field has to be clarified.

MFs (Table 3) also have different effects on bone quality. Three different kinds of stimulation were performed by applying rotating [37], pulsed [38,42], or static [47] electromagnetic fields. Both papers employing PEMF (at the same frequency of 15 Hz, but with different magnetic fields and interventions) described positive effects on the outcomes measured, thus an increased effect on bone quality coherently with a previous review on extremely low-frequency PEMF (ELF-PEMF) [52]. Rotating [37] and static [47] MFs had no significant results compared to the control group. Thus, even in this case, no trend can be underlined in session and therapy duration since, among the four included papers, [38,42] report an increased effect on bone quality, while [37,47] had no statistically significant results, although they all have a low risk of bias.

Ultrasound devices (Table 3) are the most studied, along with lasers, in the included articles (six out of 21). Ultrasound consists of mechanical energy propagating through pressure waves and representing mechanical stimulation that promotes bone formation [53,54]. In [28,30,35,40,43], sinusoidal waves were applied through periodic short bursts, with different frequencies and intensities, stimulating bone formation similarly to mechanical loading [53,55]. It is worth noting that in [28,40,43], the same device settings and duration of a single session, even compatible with those tested on humans [21], were used. Specifically, [28,40] showed not significant effects while [43] increased effects on bone quality due to measuring not notable and positive effects on BMD after applying ultrasound, respectively. This could be attributed to the longer intervention time in [43], also than [40], which shares with [43] a low risk of bias, but the few tests performed with similar intervention and setting parameters do not allow these conclusions to be drawn. Concerning the other ultrasound research papers, device settings, and intervention were quite different for [30] and [35], but the BMD measured through CBCT and DEXA, respectively, was positively affected by the treatment, enabling to deduce an increased effect on bone quality. In the remaining article [24], in which bone strengthening was achieved by causing controlled microfractures on the bones through shock waves and letting them heal, completely different settings and interventions were considered for the treatment. Also in the case of ultrasound technology, no trends can be deduced since, as well as the few papers included, similar interventions were not coupled with similar results, and research studies with similar results have different risks of bias. Indeed, [30,40,43], which share a low risk of bias, reported different effect on bone quality. Finally, [24], which has a medium risk of bias, reported mixed results.

Laser (Table 3) is the other most studied technology, with six included papers, but it has more uncertain effects than ultrasound. [30,39], showing an increased effect on bone quality due to measuring a significant positive effect on BMD, share a low risk of bias. Although the time of intervention and device settings were similar (not the devices), each session duration (50 s, the former, and 6 min, the latter) was different, bearing in mind the mean session duration for the laser technology application that is equal to 90 s. Even if similarities can be highlighted by comparing their device settings and session duration to those detected in reviewing the literature for evaluating the effectiveness of LLLT to enhance maxillofacial bone repair [23], the papers are few to draw conclusions. Low risk of bias also characterizes [33], which showed no statistically significant difference in bone quality to a control group due to measuring not notable changes in BMD. As regards the three papers showing mixed results, in [31], showing a medium risk of bias, an increased effect on bone quality resulted only in the early application stage, during the inflammation period, and it was explained through the influence of infrared laser over redox mechanisms, subsequently affecting pH levels. On the contrary, in [34], showing a high risk of bias, a slightly higher wavelength and energy density but lower power density were used compared to [31], and significant results were postponed to the consolidation period. At the same time, bone quality worsened during the latency/activation period. Finally, [32], which similarly to [34] has a high risk of bias, found no statistical significance for laser stimulation alone but an increased bone mass when applied with a mandibular propulsive appliance. As a consequence, also for laser technology, no trend for wavelengths, power intensities, or interventions can be pointed out.

In one especially compelling paper [30], ultrasound and laser technologies were compared in a randomized control trial. Two groups underwent Low-Intensity UltraSound (LIUS) and Laser (LIL) irradiation, respectively, while a third group was treated with each one on a different mandible side. A distraction device was applied to all rabbits. As reported in Table 3, both therapies resulted in an increased effect on BMD recuperation compared to the control group. Data extracted showed a more remarkable improvement in the LIUS group than in the LIL group.

Other treatments, such as the nuclear magnetic resonance (NMR) therapy — developed for the osteoporosis treatment under the brand MBST — [56–58], the application of quantum vibration intensities at multiple frequencies (QVIMF) [59], or time-varying electromagnetic fields [60] using inductive coupling (IC), among which the already mentioned PEMF [38,42], or capacitive coupling (CC) mechanisms, can be detected in the literature as relevant for human applications. Nonetheless, the literature on the topic appears to be still too immature to conduct a specific study on the subject and, therefore, these studies were not included in this analysis and are left for future investigation.

The results obtained from this review confirmed the need for a unified protocol or a methodology for assessing bone quality variations in animals. With no common measurement method, devices, and outcomes, it is complex to compare results. In the same way, different device settings (e.g., frequency and power) and interventions make it difficult to assess bone quality effectively for the same technology used and detect the appropriate stimulation mechanism. Only [30] compared the outcomes of applying two different technologies, but more is needed to draw a conclusion. In particular, standard measurement methods (CT, μCT, and CBTC), guidelines on interventions (sessions lasting a few minutes — $\simeq$ 90 s on average — for laser application, and longer — 15, up to 30, and 113 min on average, respectively — for ultrasound, mechanical vibrations, and magnetic fields application tested for different time of intervention), device settings parameters (frequencies ranging from 30 to 40 Hz in applying mechanical vibrations, of about 15 Hz for PEMF, 1.5 MHz with a 30-mW/cm^2 intensity for ultrasound, and a wavelength of 830 nm for laser technology), and animal models (mice or rats for cheaper experimental tests, and sheep for more comparable results with human) could be followed to compare outcomes achieved for each technology. Once the best combination of device settings and interventions for each technology was found, the same outcomes could be measured and compared in the case of using such device settings and session duration while varying the time of intervention.

5. Conclusions

The data analyzed in this review showed that ultrasound and laser devices are the most studied technologies, followed by MFs, mechanical vibrations, UV, and HBO. The most used animals for testing such technologies are rabbits and rats whose bone structure, however, does not share much with that of humans. Therefore, in conclusion, the possibility remains low that data extracted from this review may be valid for human bone modeling. Devices differ according to the technology considered, and commercial and custom systems were used. Each of them can be set differently by varying its characteristic parameters, and the same goes for the intervention on the animal tested since sessions range from a few seconds to some hours repeated for a different number of times and different periods. Measurements from CT and its derivatives are the most used and predictive for evaluating bone quality, usually deduced from BMD measures. Ultrasound applications were more effective in increasing bone quality, but not enough papers were found to draw a definitive conclusion on the technologies or devices that significantly affect it. Different outcomes measured, measurement methods, and interventions do not enable direct and quantitative comparison between each technology's effects on bone quality and the outcomes. Therefore, a common approach for carrying out experiments could deepen the understanding of these devices' influence on bones.

CRediT authorship contribution statement

Agostino Igor Mirulla: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing, Resources. **Chiara Brogi:** Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing, Resources. **Giuseppe Barone:** Data curation, Investigation, Methodology, Writing – review & editing, Resources. **Nicola Secciani:** Data curation, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing, Resources. **William Sansom:** Data curation, Investigation, Methodology, Writing – original draft, Resources. **Lorenzo Bartalucci:** Data curation, Investigation, Methodology, Resources. **Alessandro Ridolfi:** Conceptualization, Supervision, Visualization. **Benedetto Allotta:** Conceptualization, Methodology, Supervision, Resources. **Laura Bragonzoni:** Conceptualization, Data curation, Methodology, Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data associated with this study has not been deposited into a publicly available repository, but they are included or referenced in the article.

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