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THE EFFECT OF WHOLE BODY VIBRATION ON FRACTURE HEALING – A SYSTEMATIC REVIEW

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Abstract

This systematic review examines the efficacy and safety of whole body vibration (WBV) on fracture healing. A systematic literature search was conducted with relevant keywords in PubMed and Embase, independently, by two reviewers. Original animal and clinical studies about WBV effects on fracture healing with available full-text and written in English were included. Information was extracted from the included studies for review. In total, 19 articles about pre-clinical studies were selected. Various vibration regimes are reported; of those, the frequencies of 35 Hz and 50 Hz show better results than others. Most of the studies show positive effects on fracture healing after vibration treatment and the responses to vibration are better in ovariectomised (OVX) animals than non-OVX ones. However, several studies provide insufficient evidence to support an improvement of fracture healing after vibration and one study even reports disruption of fracture healing after vibration. In three studies, vibration results in positive effects on angiogenesis at the fracture site and surrounding muscles during fracture healing. No serious complications or side effects of vibration are found in these studies. WBV is suggested to be beneficial in improving fracture healing in animals without safety problem reported. In order to apply vibration on fractured patients, more well-designed randomised controlled clinical trials are needed to examine its efficacy, regimes and safety.

Keywords: Vibration, fracture, ovariectomy, oestrogen, systematic review.

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Introduction

Fracture is a common musculoskeletal problem with high morbidity. Most fractures usually heal uneventfully well, but about 5-10 % of the fractures result in impaired healing, such as delayed union and non-union (Rozen *et al.*, 2007). Therefore, it is of great importance to develop more efficient management to minimise delayed union and non-union. Fracture healing is a complicated biological process that consists of an inflammatory stage, repair stage and remodelling stage (Kalfas, 2001). The healing process can be influenced by various factors, of which biomechanical condition at the fracture site is considered as one of the key determinants (Einhorn, 1998; Hannouche *et al.*, 2001). As mechanical stimulation can optimise the structural properties of the fracture (Duncan and Turner, 1995) and, also, sensitise different cells to differentiate in the direction of bone repair (Wei *et al.*, 2016), early phases of fracture healing may respond well to vibration. In recent years, osteocytes have been identified as being responsible for the perception and regulation of mechanical effects on the skeleton through various mechanotransduction mechanisms including ion channels, integrins, primary cilia, *etc.* (Yavropoulou and Yovos, 2016). There is a need for new non-invasive mechanical stimulation therapies for fracture healing.

Vibration is a form of mechanical stimulation that provides a physical oscillation (Cardinale and Wakeling, 2005; Rittweger, 2010). Parameters, such as peak-to-peak displacement (or amplitude), frequency and acceleration of the oscillation are used to describe

a vibration, as recommended by the International Society of Musculoskeletal and Neuronal Interactions (ISMNI) (Rauch *et al.*, 2010). These parameters are also main factors that determine the intensity of vibration (Cardinale and Bosco, 2003). Whole body vibration (WBV) generates high-frequency mechanical stimuli transferred to the whole body (Gomez-Cabello *et al.*, 2014). Some studies provide evidence that WBV has effects on the musculoskeletal system, including improving muscle function (Rees *et al.*, 2008; Sitja-Rabert *et al.*, 2015), increasing bone mineral density (BMD) (Lam *et al.*, 2013; Verschueren *et al.*, 2004), reducing risks of falls and improving muscle strength and balancing ability (Leung *et al.*, 2014). Low-magnitude high-frequency vibration (LMHFV), a type of WBV with magnitude usually lower than 1 ×*g* [magnitude, in gravitational acceleration (m/s^2)] and frequency ranged from 20 to 90 Hz (Bemben *et al.*, 2010; Fuermaier *et al.*, 2014; von Stengel *et al.*, 2011), is also reported to have positive effects on osteogenic differentiation and osteogenesis (Dumas *et al.*, 2010; Kim *et al.*, 2012; Leung *et al.*, 2014; Luu *et al.*, 2009). Some previous studies using LMHFV for fracture healing in rats report its promising effects on callus formation, mineralisation and bone remodelling (Chow *et al.*, 2011; Leung *et al.*, 2009; Shi *et al.*, 2010). However, some studies reveal that bone metabolism (Bemben *et al.*, 2010) and fracture healing process (Wolf *et al.*, 2001) are not influenced by vibration treatment.

In spite of an increasing number of studies about vibration treatment for fracture healing, the results are variable and the efficacy of vibration on fracture healing remains unclear. Therefore, a systematic review was conducted aiming to analyse the efficacy and safety of vibration treatment on fracture healing, which may serve as a reference for planning of clinical trials.

Materials and Methods

Search strategy

Literature search was performed on PubMed and Embase (last access to both was on 12th March 2017). The keywords included were vibration, WBV, LIV (low intensity vibration), LMHFV and fracture. We combined the keywords as vibration **OR** WBV **OR** LIV **OR** LMHFV **AND** fracture and searched in all fields. This search strategy was used for both databases.

Search criteria

Inclusion criteria were: 1) pre-clinical or clinical studies that investigated the effects of vibration on fracture healing process, with a control group; 2) studies that had outcomes related to bone morphology and/or function; 3) full-text literature published in English.

Exclusion criteria were: 1) non-English-language papers; 2) not vibration treatment-related; 3) not fracture-related; 4) review articles; 5) reports published as conference abstracts.

Selection of studies

Study selection was conducted by two reviewers, independently. Primary screening of titles and abstracts was performed to exclude obviously irrelevant papers. Then, potential relevant articles were retrieved and reviewed with the inclusion and exclusion criteria. Disagreements were resolved by discussion and consensus.

Data extraction

The following information was extracted by reviewers: methodology, fracture models used, species, sample size, regime of vibration (frequency, acceleration), radiographic results, histomorphometric results, angiogenesis findings, gene expression analysis and all the related data on efficacy and safety.

Data analysis

As the studies included in this review were all pre-clinical studies and there was variability in terms of animal species, methodological and statistical heterogeneity. For this reason, it was inappropriate to combine the studies to conduct a meta-analysis. Hence, only qualitative review was conducted. The analysis of the effect of oestrogen on vibration treatment was presented in bubble chart using Numbers (Apple, Cupertino, CA, USA).

Results

Results of the search

After the initial search, the total number of papers was 351 from PubMed and 510 from Embase database. After reviewing titles and abstracts of the 861 papers, most of them were excluded based on the selection criteria and 27 of them were identified as potentially eligible for further examination. After screening the papers in detail, 8 more articles were excluded, out of which 4 were conference reports, with one focusing on incisor extraction but not fracture, one adopting bone defect model, one not using vibration treatment and one investigating implant osseointegration. Therefore, 19 manuscripts were finally recruited for analysis (Bilgin *et al.*, 2017; Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Gao *et al.*, 2016; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Leung *et al.*, 2009; Shi *et al.*, 2010; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Uchida *et al.*, 2017; Usui *et al.*, 1989; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014; Wei *et al.*, 2016; Wolf *et al.*, 2001). The flow diagram in Fig. 1 summarises the selection process.

Characteristics of the included studies

The 19 selected studies were conducted between 1989 and 2017. All of them are pre-clinical studies: fifteen rat studies (Bilgin *et al.*, 2017; Butezloff *et al.*, 2015;

Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Gao *et al.*, 2016; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Leung *et al.*, 2009; Shi *et al.*, 2010; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Uchida *et al.*, 2017; Wei *et al.*, 2016), two mouse studies (Wehrle *et al.*, 2015; Wehrle *et al.*, 2014), one sheep study (Wolf *et al.*, 2001) and one rabbit study (Usui *et al.*, 1989). No clinical study was found, based on our search criteria on publications prior to the 12th March 2017. Among the fifteen rat studies, one uses males (Bilgin *et al.*, 2017). Among the other fourteen studies on female rats, five (Chow *et al.*, 2011; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Wei *et al.*, 2016) report vibration treatment on ovariectomised (OVX) rats, five (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2016; Chung *et al.*, 2014; Shi *et al.*, 2010) apply vibration to both OVX and sham-OVX models and one to both OVX and non-OVX rats (Stuermer *et al.*, 2010). Three studies (Gao *et al.*, 2016; Leung *et al.*, 2009; Uchida *et al.*, 2017) use only non-OVX rats as research subjects. Among the two mouse studies, one (Wehrle *et al.*, 2015) investigate the effects of vibration on both OVX and sham-OVX mice and the other (Wehrle *et al.*, 2014) uses non-OVX models only. Both, the sheep study (2-3 years old female sheep) and the rabbit study (adult female rabbit) do not report the use of an osteoporosis/osteopenia model (Table 1**A**,**B**,**C**).

In all nineteen included studies, fractures are created in the animals. Nine of them use closed fracture models with Kirschner wire (K-wire) internal fixation; of those, eight are carried out on the femur of the animals (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Leung *et al.*, 2009; Shi *et al.*, 2010; Wei *et al.*, 2016) and one uses the tibia fracture model (Gao *et al.*, 2016). The other ten studies include two femoral fracture models (Wehrle *et al.*, 2015; Wehrle *et al.*, 2014), five tibial fracture models (Bilgin *et al.*, 2017; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010), one metatarsal fracture model [in which researchers conducted osteotomy with external fixators or titanium plates (Wolf *et al.*, 2001)], one rib fracture model without fixation (Uchida *et al.*, 2017) and one fibular osteotomy model without fixation (Usui *et al.*, 1989). Fifteen diaphyseal fracture models and four metaphyseal fracture models are used in the included studies (Table 1**A**,**B**,**C**).

Diversified vibration regimes were found in the included articles, including frequency, amplitude and magnitude. A summary of the vibration regimes is shown in Table 2**A**,**B**. The vibration frequency ranges from 20 Hz to 90 Hz and amplitude from 0.02 mm to 1 mm. The relationship among frequency, amplitude and magnitude can be expressed in the following equation (Eq. 1):

$$g = \frac{A(2\pi f)^2}{9.81}$$

where g represents the magnitude [in gravitational acceleration (m/s²)], A the amplitude (in m) and f the frequency (in Hz). The treatment duration ranges from 10 to 60 min/d, with most of the treatments lasting 20 min/d.

Outcomes (Table 3)

Vibration regime

Many vibration regimes are found in the included articles, with amplitudes ranging from 0.02 mm to

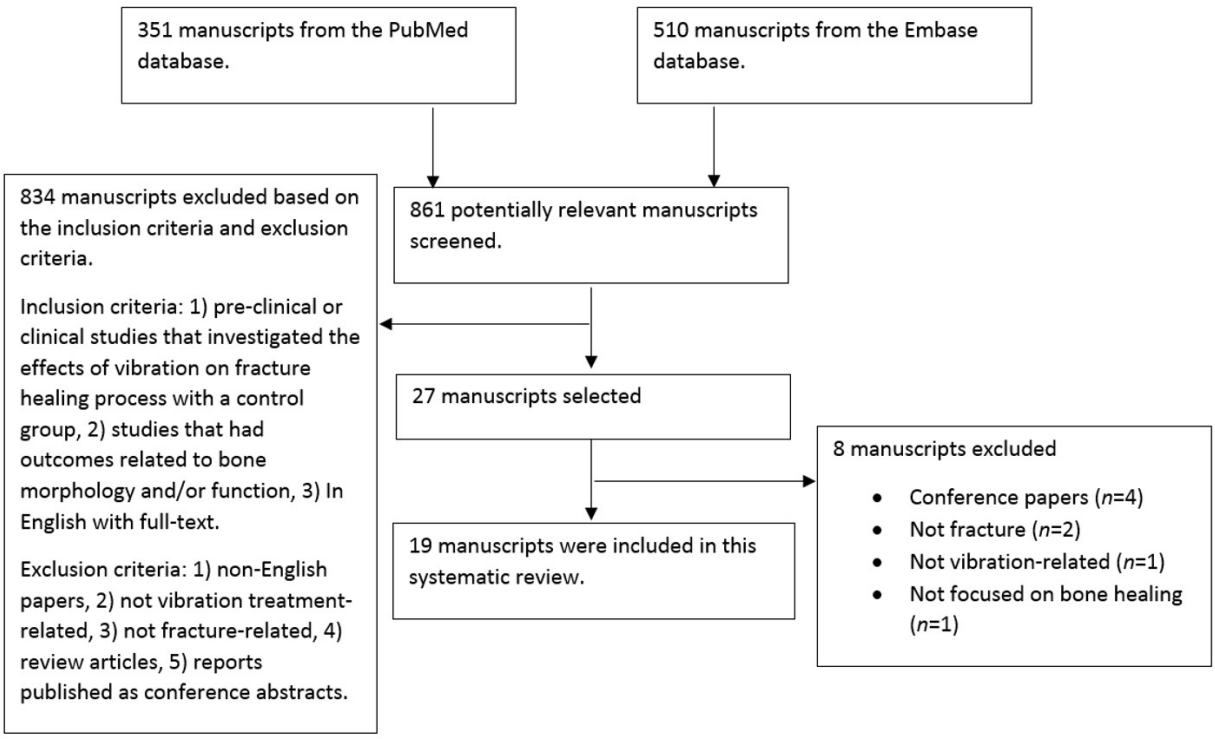

Fig. 1. Flow diagram showing the process of literature search.

Table 1A. Summary of the study characteristics. Abbreviations: OF: open fracture; CF: closed fracture; PEMF: pulsed electromagnetic fields; OVX: ovariectomised; ER: oestrogen receptor; VT: vibration treatment; SD: Sprague-Dawley; FBC: fractured model without mechanical loading; PTH: parathyroid hormone; SR: strontium ranelate.

Included studies	Study design	Species	Strain	Age	Fracture site	Fracture model	Fixation type	OVX or not	Study duration
Bilgin *et al.*, (2017)	Group 1: control (n = 10); group 2: VT (n = 10); group 3: PEMF (n = 10).	Rat (male)	SD	3 months	Tibia (diaphysis)	OF	Intramedullary pinning	N/A	28 d
Butezloff *et al.*, (2015)	Group 1: control (n = 14); group 2: control + OVX (n = 14); group 3: VT (n = 14); group 4: VT + OVX (n = 14).	Rat (female)	Wistar	5 months	Femur (diaphysis)	CF	Internal (K wire)	OVX + sham-OVX	28 d
Cheung *et al.*, (2012)	Group 1: sham-control (n = 18); group 2: sham-VT (n = 18); group 3: OVX-control (n = 18); group 4: OVX-VT (n = 18).	Rat (female)	SD	9 months	Femur (diaphysis)	CF	Internal (K-wire)	OVX + sham-OVX	56 d
Chow *et al.*, (2016)	Group 1: sham-control; group 2: sham-VT; group 3: OVX; group 4: OVX-VT; group 5: OVX-VT-ICI (VT: whole-body VT, ICI: ER antagonisation by ICI 182,780; n = 5 and n = 4 per group per time point for gene expression and μ-CT assessments, respectively).	Rat (female)	SD	9 months	Femur (diaphysis)	CF	Internal (K-wire)	OVX + sham-OVX	8 weeks
Chow *et al.*, (2011)	Group 1: OVX-control (n = 20); group 2: OVX + VT (n = 20); group 3: OVX + bisphosphonate (n = 20); group 4: OVX + VT + bisphosphonate (n = 20).	Rat (female)	SD	9 months	Femur (diaphysis)	CF	Internal (K-wire)	OVX	56 d
Chung *et al.*, (2014)	Group 1: sham-control (n = 36); group 2: sham-VT (n = 36); group 3: OVX-control (n = 36); group 4: OVX-VT (n = 36).	Rat (female)	SD	9 months	Femur (diaphysis)	CF	Internal (K wire)	OVX + sham-OVX	56 d
Gao *et al.*, (2016)	Group 1: DL (VT 15 min/d); group 2: DLR (3 bouts of 5 min of VT were separated by 4 h); group 3: VL7 [VT for 7 d (15 min/d), followed by a 7 d rest period]; group 4: VL7R [VT for 7 d (3 bouts of 5 min separated by 4 h), followed by a 7 d rest period]; group 5: FBC; n = 7 in each group.	Rat (female)	N/A	3 months + 2 weeks	Tibia (diaphysis)	CF	Internal (K wire)	non-OVX	4 weeks
Komrakova *et al.*, (2016)	Group 1: non-OVX; group 2: OVX-no treatment; group 3: OVX + VT; group 4: SR; group 5: SR + VT; group 6: PTH; group 7: PTH + VT (n = 12 in each group).	Rat (female)	SD	5 months	Tibia (metaphysis)	OF	Internal (titanium plate)	OVX + non-OVX	42 d

Table 1B. Summary of the study characteristics. Abbreviations: OF: open fracture; CF: closed fracture; OVX: ovariectomised; VT: vibration treatment; SD: Sprague-Dawley; WBV: whole body vibration.

Included studies	Study design	Species	Strain	Age	Fracture site	Fracture model	Fixation type	OVX or not	Study duration
Komrakova *et al.*, (2013)	Experiment 1. Group 1: control (n = 15); group 2: OVX (n = 15); group 3: OVX + 35 Hz vertical VT (n = 15); group 4: OVX + 50 Hz vertical VT (n = 15); group 5: OVX + 70 Hz vertical VT (n = 15); group 6: OVX + 90 Hz vertical VT (n = 15). Experiment 2. Group 1: control (n = 15); group 2: OVX (n = 15); group 3: OVX + 30 Hz horizontal VT (n = 15); group 4: OVX + 50 Hz horizontal VT (n = 15); group 5: OVX + 70 Hz horizontal VT (n = 15); group 6: OVX + 90 Hz horizontal VT (n = 15).	Rat (female)	SD	5 months	Tibia (metaphysis)	OF	Internal (titanium plate)	OVX	35 d
Leung *et al.*, (2009)	Group 1: control (n = 26), group 2: VT (n = 26).	Rat (female)	SD	3 months	Femur (diaphysis)	CF	Internal (K-wire)	non-OVX	28 d
Shi *et al.*, (2010)	Group 1: OVX-control (n = 26); group 2: OVX-VT (n = 26); group 3: control (n = 26); group 4: VT (n = 26).	Rat (female)	SD	9 months	Femur (diaphysis)	CF	Internal (K-wire)	OVX + sham-OVX	56 d
Stuermer *et al.*, (2010)	Group 1: intact non-VT (n = 15); group 2: intact-VT (n = 15); group 3: OVX-non-VT (n = 15); group 4: OVX-VT (n = 15).	Rat (female)	SD	22 weeks	Tibia (metaphysis)	OF	Internal (titanium plate)	OVX + non-OVX	35 d
Stuermer *et al.*, (2014)	Group 1: intact (n = 12); group 2: OVX (n = 12); group 3: OVX + WBV (n = 12); group 4: OVX + estradiol (n = 12); group 5: OVX + VT + estradiol (n = 12); group 6: OVX + raloxifene (n = 12); group 7: OVX + VT + raloxifene (n = 12).	Rat (female)	SD	5 months	Tibia (metaphysis)	OF	Internal (titanium plate)	OVX	42 d

Table 1C. Summary of the study characteristics. Abbreviations: OF: open fracture; CF: closed fracture; OVX: ovariectomised; E2: oestrogen; VT: vibration treatment; SD: Sprague-Dawley; MSC: mesenchymal stem cells.

Included studies	Study design	Species	Strain	Age	Fracture site	Fracture model	Fixation type	OVX or not	Study duration
Uchida *et al.*, (2017)	Vibration acceleration: control-VA (n = 8); low (n = 8); high (n = 8). Constant acceleration: control-CA (n = 8); centrifuge (n = 8).	Rat (female)	Wistar	4 weeks	Rib (diaphysis)	OF	N/A	Non-OVX	9 d
Usui *et al.*, (1989)	Study 1. Group 1: control (n = 12); group 2: VT (n = 12). Study 2. Group 1: control (n = 3); group 2: VT (n = 4). Study 3. Group 1: control (n = 7); group 2: VT (n = 8). Study 4. Group 1: control (n = 3); group 2: VT (n = 4).	Rabbit (female)	New Zealand White	Adult	Fibula (diaphysis)	OF	N/A	non-OVX	42 d
Wehrle *et al.*, (2014)	Group 1: control (n = 20); group 2: 35 Hz VT (n = 14); group 3: 45 Hz VT (n = 14).	Mouse (female)	C57BL/6NCrl	12 weeks	Femur (diaphysis)	OF	External (3 N/mm)	non-OVX	21 d
Wehrle *et al.*, (2015)	Group 1: non-OVX, non-LMHFV (n = 19); group 2: non-OVX, LMHFV (n = 21); group 3: OVX, non-LMHFV (n = 21); group 4: OVX, LMHFV (n = 20); group 5: OVX, E2, non-LMHFV (n = 8); group 6: OVX, E2, LMHFV (n = 8).	Mouse (female)	C57BL/6NCrl	49 weeks	Femur (diaphysis)	OF	External (3N/mm)	OVX+ sham-OVX	21 d
Wei *et al.*, (2016)	Group 1: VAMG (VT + AMD3100 + MSC); group 2: VMG (VT + MSC); group 3: MG (MSC); group 4: CG (control) [n = 10 for green fluorescent protein (GFP) signal and μ-CT assessments and n = 8 for mechanical test].	Rat (female)	SD	9 months	Femur (diaphysis)	CF	Internal (K-wire)	OVX	8 weeks

5 mm and magnitude from 0.03 ×g to 16.3 ×g. As shown in Table 2**A**,**B**, the frequencies range from 20 Hz to 90 Hz, with many studies (8 out of 19) using a frequency of 35 Hz. Two studies report a comparison of the effects of different frequencies (Komrakova *et al.*, 2013; Wehrle *et al.*, 2014) and one study compares low and high magnitude vibration during fracture healing (Uchida *et al.*, 2017). Most studies (12 out of 19) report starting vibration treatment at day 5 postoperatively, four studies commence at day 3 post-fracture, two studies at day 7 and day 14 respectively and the final study does not report the exact start time. Also, treatment duration varies among these studies. Ten (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Leung *et al.*, 2009; Shi *et al.*, 2010; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014; Wei *et al.*, 2016) of the nineteen studies adopt a 20 min/d treatment, four (Bilgin *et al.*, 2017; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010) a 15 min/d treatment, one (Wolf *et al.*, 2001) 5 min/d, one 15 min 2×/d and one (Usui *et al.*, 1989) compares 20 min/d and 60min/d vibration treatment during fracture healing. One study (Gao *et al.*, 2016) applies four different regimes: 1) 15 min/d; 2) three bouts of 5 min of vibration separated by 4 h; 3) 15 min/d, 7 d, followed by a 7 d rest; 4) 7 d followed by a 7 d rest, with three bouts of 5 min of vibration separated by 4 h. This study compares different rest period regimes in fracture healing. Besides treatment duration per day, the treatment per week and total treatment period also varies in the nineteen studies. The detailed regime and vibration duration of each study are listed in Table 2**A**,**B**.

In this review, vibration regimes with a magnitude lower than 1 ×*g* were regarded as LMHFV and those with a magnitude greater than 1 ×*g* were regarded as HMHFV (high magnitude high frequency vibration). Six (Butezloff *et al.*, 2015; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Usui *et al.*, 1989) of the included studies use HMHFV and twelve (Bilgin *et al.*, 2017; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Gao *et al.*, 2016; Leung *et al.*, 2009; Shi *et al.*, 2010; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014; Wei *et al.*, 2016; Wolf *et al.*, 2001) adopt LMHFV. One study uses both LMHFV and HMHFV (Uchida *et al.*, 2017).

Komrakova *et al.* (2013) (HMHFV, 35-90 Hz, 2.5-16.3 ×*g*, open fracture, rat) report the influence of different vibration frequencies on fracture healing. This study uses a metaphyseal osteotomy model of the tibia, with titanium plate fixation to evaluate the effects of both vertical and horizontal HMHFV on the healing. In vertical HMHFV groups, micro–computed tomography (μCT) and microradiography findings show that vibration treatment improve fracture healing with respect to cortical density, callus density, callus width (CW) and first osseous bridging (35 and 50 Hz). More specifically, 35 Hz and 50 Hz vertical vibration accelerate osseous bridging and improve callus formation and cortical density more than other frequencies. Vertical vibration at 70 Hz and 90 Hz improve some callus parameters and related gene expression, but the osteotomy bridging is not accelerated. However, the horizontal vibration reduces the cortical width at the ventral area and, at values below 90 Hz, the callus density in the dorsal area. The dorsal callus area (CA) is reduced by 90 Hz vibration, while the endosteal callus area is increased by all vertical vibration.

Other bone healing parameters are not affected by a horizontal vibration of 90 Hz. Wehrle *et al.* (2014) (LMHFV, 35-45 Hz, 0.3 ×*g*, open fracture, mouse) also compare the effects of different frequencies of vibration treatment on fracture healing. They start the vibration treatment on the third day after fracture and the mice are subjected to 20 min/d of vibration treatment, 5 d per week. The mice are sacrificed 10 d or 21 d after surgery. Mechanical testing (day 21), histomorphometry (day 10 and 21) and μCT analyses (day 21) indicate that no significant difference in flexural rigidity, callus size and relative tissue amounts at the fracture site are present between 35 Hz and no-treatment control groups. In contrast, flexural rigidity of the 45 Hz vibration-treatment group is significantly reduced compared to the no-treatment control and a decreasing trend of callus volume and BV/TV (trabecular bone volume fraction) are observed, although not significantly different.

10 out of 19 studies considered in this review adopt a 20 min/d vibration duration, 9 (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Leung *et al.*, 2009; Shi *et al.*, 2010; Wehrle *et al.*, 2015; Wei *et al.*, 2016) of which show positive effects on fracture healing. Usui *et al.* (1989) [HMHFV, 25 Hz, two dimensional, 1.7 ×*g* (horizontal), 2.0 ×*g* (vertical), open fracture, rabbit] report on both 20 and 60 min/d two-dimensional vibration treatment during fracture healing. They find that callus size and mineralised callus area are increased significantly in the 60 min vibration-treatment group. The 20 min group reveal a slight increases in callus and mineralised callus areas without significant difference. Gao *et al.* (2016) describe the influences of different vibration regimes: 1) 15 min/d; 2) 3 bouts of 5 min of vibration separated by 4 h; 3) 15 min/d, 7 d followed by a 7 d rest; 4) 7 d followed by a 7 d rest, with 3 bouts of 5 min of vibration separated by 4 h. The DLR group (vibrational loading per day in which three bouts of 5 min of vibration are separated by 4 h) show a great potential for clinical practices.

Radiography and μCT *analysis*

Radiography and/or μCT assessments at the fracture site are reported in all the included studies (Table 3). Generally, nine closed fracture studies (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Gao *et al.*, 2016; Leung *et al.*, 2009; Shi *et al.*, 2010; Wei *et al.*, 2016) and seven (Bilgin *et al.*, 2017; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Uchida *et al.*, 2017; Usui *et al.*, 1989; Wehrle *et al.*, 2015; Wolf *et al.*, 2001) of the ten open fracture

Table 2A. Summary of vibration regimes. * All parameters not shown in articles were calculated by Eq. 1. ** Vertical vibration, unless otherwise

Included studies	Type of vibration	Amplitude (mm)**	Frequency(Hz)	Magnitude (×*g*)*	Start time	Duration
Bilgin *et al.*, (2017)	LMHFV	0.03*	50	0.35	5 d after fracture	15 min/d, 7 d/week, lasted 3 weeks
Butezloff *et al.*, (2015)	HMHFV	0.5	60	7.2*	3 d after fracture	20 min/d, 3 d/week, end at day 14 or 28 post-fracture
Cheung *et al.*, (2012)	LMHFV	0.06*	35	0.3	5 d after fracture	20 min/d, 5 d/week, 2/4/8 weeks
Chow *et al.*, (2016)	LMHFV	0.06*	35	0.3	5 d after fracture	20 min/d, 5 d/week, 2/4/8 weeks
Chow *et al.*, (2011)	LMHFV	0.06*	35	0.3	5 d after fracture	20 min/d, 5 d/week, 2/4/6/8 weeks
Chung *et al.*, (2014)	LMHFV	0.06*	35	0.3	5 d after fracture	20 min/d, 5 d/week, 2/4/8 weeks
Gao *et al.*, (2016)	LMHFV	0.05*	35	0.25	1 week after fracture	1) 15 min/d; 2) 3 bouts of 5 min of vibration separated by 4 h rest time; 3) 15 min/d, 7 d followed by a 7 d rest; 4) 7 d followed by a 7 d rest, with 3 bouts of 5 min of vibration separated by 4 h, for 4 weeks.
Komrakova *et al.*, (2016)	HMHFV	0.5	70	3	5 d after fracture	15 min twice a day, for 37 d
Komrakova *et al.*, (2013)	HMHFV	0.5 (vertical/ horizontal)	Vertical: 35, 50, 70 and 90; horizontal: 30, 50, 70 and 90	Vertical: 2.5, 5.0, 9.9 and 16.3; horizontal: 1.8, 5.0, 9.9 and 16.3*	5 d after fracture	15 min/d, daily, for 30 d
Leung *et al.*, (2009)	LMHFV	0.06*	35	0.3	5 d after fracture	20 min/d, 5 d/week, 1/2/4 weeks
Shi *et al.*, (2010)	LMHFV	0.06*	35	0.3	5 d after fracture	20 min/d, 5 d/week, 2/4/8 weeks
Stuermer *et al.*, (2010)	HMHFV	0.5	90	4	5 d after fracture	15 min twice a day, daily, for 30 d

Table 2B. Summary of vibration regimes. * All parameters not shown in articles were calculated by Eq. 1. ** Vertical vibration, unless otherwise specified. † This study used their own definition of LMHFV (-1.5 to 1.5 ×*g*) and HMHFV (-3 to 3 ×*g*).

Included studies	Type of vibration	Amplitude (mm)**	Frequency (Hz)	Magnitude (×*g*)*	Start time	Duration
Stuermer *et al.*, (2014)	HMHFV	0.4	70	7.9	5 d after fracture	15 min twice a day, daily, 6 weeks
Uchida *et al.*, (2017)	LMHFV/HM-HFV	2.5/5	30	-1.5 to 1.5; -3 to 3 †	3 d after fracture	10 min/d, for 9 d
Usui *et al.*, (1989)	HMHFV	Two dimensional: 0.67 (horizontal); 0.8 (vertical)*	25	1.7 (horizontal); 2.0 (vertical)	N/A	20 min/d or 60 min/d, daily, end at week 3 or week 6.
Wehrle *et al.*, (2014)	LMHFV	0.04/0.06*	45/35	0.3	3 d after fracture	20 min/d, 5 d/ week, 10 or 21 d
Wehrle *et al.*, (2015)	LMHFV	0.04*	45	0.3	3 d after fracture	20 min/d, 5 d/ week, 10 or 21 d
Wei *et al.*, (2016)	LMHFV	0.06*	35	0.3	5 d after fracture	20 min/d, 5 d/ week, 2/4/8 weeks
Wolf *et al.*, (2001)	LMHFV	0.02	20	0.03*	2 weeks after fracture	5 min/d, 5 d/week, end at week 8

studies (Bilgin *et al.*, 2017; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Uchida *et al.*, 2017; Usui *et al.*, 1989; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014; Wolf *et al.*, 2001) present positive results, yet three open fracture studies (Komrakova *et al.*, 2016; Stuermer *et al.*, 2010; Wehrle *et al.*, 2014) show no improvement due to vibration treatment in radiography and analysis.

All the closed fracture studies (9 out of 19) use internal fixation with K-wire. Five of the nine closed fracture studies including Leung *et al.* (2009) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat), Shi *et al.* (2010) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat), Chow *et al.* (2011) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat), Chung *et al.* (2014) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat) and Chow *et al.* (2016) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat) show a faster bridging of fracture gaps and larger CW and CA in vibration-treatment groups. Significantly higher BV (trabecular bone volume) and BV/TV are found in the vibration-treatment groups in three studies conducted by Leung *et al.* (2009), Shi *et al.* (2010) and Butezloff *et al.* (2015) (HMHFV, 60 Hz, 14.4 ×*g*, closed fracture, rat). Cheung *et al.* (2012) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat) also report significantly larger CW, CA, BV and tissue volume (TV) in the vibration-treatment groups. Butezloff *et al.* (2015) (HMHFV, 60 Hz, 14.4 ×*g*, closed fracture, rat) show that BMD (bone mineral density), BMC (bone mineral content), bone callus density and callus volume are significantly increased in the vibration-treatment groups. Chow *et al.* (2011) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat), describe higher LD-BV/TV (low density bone volume fraction) in the vibration-treatment group (sham-OVX) than in the control samples. Similar results, but without statistical significance, are found in the OVX-vibration and OVX-control groups. Wei *et al.* (2016) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat) present the positive results of combined treatment of vibration + mesenchymal stem cells (MSC). CW, CA, high-density bone volume (BVh), BV/TV and BMD in vibration + MSC are significantly higher than in the other 3 groups. Gao *et al.* (2016) (LMHFV, 35 Hz, 0.25 ×*g*, closed fracture, rat) report that tissue mineral density (TMD) of vibration-treatment group is significantly higher compared to control group.

Five (Bilgin *et al.*, 2017; Komrakova *et al.*, 2013; Uchida *et al.*, 2017; Usui *et al.*, 1989; Wehrle *et al.*, 2015) of the seven open fracture studies show significant differences and two (Stuermer *et al.*, 2014; Wolf *et al.*, 2001) show no significant differences. Usui *et al.* (1989) [HMHFV, 25 Hz, two dimensional, 1.7 ×*g* (horizontal), 2.0 ×*g* (vertical), open fracture, rabbit] demonstrate a significant increase of callus size in the vibration-treatment group. Cortical density and CW are significantly higher in the vibration-treatment group in Komrakova *et al.* (2013) study (HMHFV, 35-90 Hz, 2.5-16.3 ×*g*, open fracture, rat) and larger BV/TV was found in Wehrle and colleagues study (LMHFV, 45 Hz, 0.3 ×*g*, open fracture, mouse) (Wehrle *et al.*, 2015). In Bilgin *et al.* (2017) (LMHFV, 50 Hz, 0.35 ×*g*, open fracture, rat), the total summed area of new bone, cartilage summed area and trabecular summed area are significantly larger in vibration-treatment groups than control rats. Callus formation, fracture line and fracture gap bridging in the vibration-treatment group show a trend towards improvement, although without significant differences. Uchida *et al.* (2017) (LMHFV/HMHFV, 35 Hz, 1.5-3.5 ×*g*, open fracture, rat) report that union rate, BV and BV/TV in the vibration-treatment group are also significantly higher than in the control group.

Table 3. Summary of outcomes in response to vibration treatments.

Included studies		Radiography/μ-CT		Histomorphometry		Mechanical testing		Gene expression
		Positive outcome	Significant difference	Positive outcome	Significant difference	Positive outcome	Significant difference	
Bilgin *et al.*, (2017)		✓	✓	×	×			
Butezloff *et al.*, (2015)	Day 14	✓	✓			✓	×	
	Day 28	✓	✓			✓	×	
Cheung *et al.*, (2012)	Day 14	✓	✓	✓	✓			
	Day 28	✓	✓	✓	✓			
	Day 56	×	×	×	×			
Chow *et al.*, (2016)	Day 14	✓	✓					
	Day 28	✓	✓					
	Day 56	✓	✓					
Chow *et al.*, (2011)	Day 14	×	×					
	Day 28	✓	✓	×	×			
	Day 42	✓	✓	✓	✓			
	Day 56	✓	✓	×	×			
Chung *et al.*, (2014)	Day 14	✓	✓	✓	✓			Col-2, Col-1, RANKL, OPG
	Day 28	✓	✓	✓	✓			
	Day 56	✓	✓	✓	✓			
Gao *et al.*, (2016)		✓	✓			✓	✓	
Komrakova *et al.*, (2016)		×	×			×	×	ALP, Oc, TRAP, RANKL, OPG
Komrakova *et al.*, (2013)		✓	✓	✓	✓	×	×	ALP, Oc, RANKL, OPG, TRAP
Leung *et al.*, (2009)	Day 7	✓	✓	✓	✓			
	Day 14	✓	✓	✓	✓			
	Day 28	✓	✓			✓	✓	
Shi *et al.*, (2010)	Day 14	✓	✓	×	×			
	Day 28	✓	✓	✓	×			
	Day 56	✓	✓	×	×	✓	✓	
Stuermer *et al.*, (2010)		×	×	✓	✓	×	×	ALP, Oc, TRAP-1, IGF-1
Stuermer *et al.*, (2014)		✓	×	✓	✓	✓	×	ALP, Oc, RANKL, OPG, TRAP, ER-α, IGF-1
Uchida *et al.*, (2017)		✓	✓	✓				
Usui *et al.*, (1989)	Day 21	✓	✓	✓	✓	✓	×	
	Day 42	×	×			✓	×	
Wehrle *et al.*, (2014)	Day 10			×	×			
	Day 21	×	×	×	×	×	×	
Wehrle *et al.*, (2015)	Day 10			✓	×			Esr2, Esr1, Tnfrsf11B, Tnsf11, Sost, β-catenin, cmyc, Bglap, Spp1, Col2a1, Sox9
	Day 21	✓	✓	✓	✓	✓	✓	
Wei *et al.*, (2016)	Day 14	✓	✓					
	Day 28	✓	✓					
	Day 56	✓	✓			✓	✓	
Wolf *et al.*, (2001)		✓	×			×	×	

Cross-sectional area, BMD and BMC are slightly higher in the vibration-treatment group, but without significant differences according to Wolf *et al.* (2001) (LMHFV, 20 Hz, 0.03 ×*g*, open fracture, sheep) and the increases of BMD, BV, BV/TV in the vibration-treatment group are also not significant in Stuermer *et al.*(2014) (HMHFV, 70 Hz. 7.9 ×*g*, open fracture, rat). Stuermer *et al.* (2010) (HMHFV, 90 Hz, 4 ×*g*, open fracture, rat) and Wehrle *et al.* (2014) (LMHFV, 45/35 Hz, 0.3 ×*g*, open fracture, mouse) show no significant differences during fracture healing in radiography and μCT analysis between vibration and control groups. Similarly, Komrakova *et al.* (2013) (HMHFV, 70 Hz. 0.3-3 ×*g*, open fracture, rat) report that vibration alone does not change the radiography and μCT parameters.

8 of the 10 open fracture models use intramedullary pinning (Bilgin *et al.*, 2017), titanium plate (Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010), 3 N/mm external fixator (Wehrle *et al.*, 2015; Wehrle *et al.*, 2014) and 1160 N/mm external fixator (Wolf *et al.*, 2001) as fixation, while the resting two open fracture studies use the natural skeletal structure instead of external fixation (Uchida *et al.*, 2017; Usui *et al.*, 1989). Two (Komrakova *et al.*, 2016; Stuermer *et al.*, 2010) of the four titanium plate fixation studies and one (Wehrle *et al.*, 2014) of the three external fixator studies present negative results for the radiography and μCT analysis. Another titanium plate fixation study (Stuermer *et al.*, 2014) and an external fixator study (Wolf *et al.*, 2001) report a positive trend for the radiography and μCT analysis, but with no statistically significant difference.

As shown in Table 2**A**,**B** and Table 3, various treatment duration and corresponding outcomes are used in the included 19 studies. Around week 2 post-fracture (fibrous tissue formation stage), the radiology and/or μCT assessments of nine studies (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2016; Chung *et al.*, 2014; Leung *et al.*, 2009; Shi *et al.*, 2010; Uchida *et al.*, 2017; Usui *et al.*, 1989; Wei *et al.*, 2016) show positive outcomes, while Chow *et al.* (2011) find no significant difference between vibration and control groups. 3 weeks post-fracture (hard callus/secondary bone formation stage), Bilgin *et al.* (2017) and Wehrle *et al.* (2015) show better results in the vibration-treatment groups compared to control, while according to Wehrle *et al.* (2014), the vibration-treatment group does not present significant differences in μCT assessments as compared to control. Komrakova *et al.* (2016), Wei *et al.* (2016), Chow *et al.* (2016), Gao *et al.* (2016), Chung *et al.* (2014), Stuermer *et al.* (2014), Komrakova *et al.* (2013), Cheung *et al.* (2012), Chow *et al.* (2011), Stuermer *et al.* (2010), Shi *et al.* (2010), Leung *et al.* (2009), Wolf *et al.* (2001) and Usui *et al.* (1989) report radiology and/or μCT assessments at the bone remodelling stage (after day 21). At this time point, Komrakova *et al.* (2016), Stuermer *et al.* (2010) and Usui *et al.* (1989) find negative outcomes in the vibration-treatment groups as compared to the control groups. The other studies present positive results of vibration treatment at the remodelling stage.

Histomorphology analysis

Histomorphology analysis are reported in thirteen (Bilgin *et al.*, 2017; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chung *et al.*, 2014; Komrakova *et al.*, 2013; Leung *et al.*, 2009; Shi *et al.*, 2010; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Uchida *et al.*, 2017; Usui *et al.*, 1989; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014) of the included articles; five (Cheung *et al.*, 2012; Chow *et al.*, 2011; Chung *et al.*, 2014; Leung *et al.*, 2009; Shi *et al.*, 2010) of which are closed fracture studies and eight (Bilgin *et al.*, 2017; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Uchida *et al.*, 2017; Usui *et al.*, 1989; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014) open fracture studies.

In the five closed fracture studies, four (Chow *et al.*, 2011; Chung *et al.*, 2014; Leung *et al.*, 2009; Shi *et al.*, 2010) show positive effects of vibration treatment on fracture healing, including three (Chow *et al.*, 2011; Chung *et al.*, 2014; Leung *et al.*, 2009) studies with significant differences and one (Shi *et al.*, 2010) without significant difference. The other study evaluated vascular endothelial growth factor (VEGF) expression and is discussed later (Cheung *et al.*, 2012). Significantly larger TCA (total callus area), CCA (cartilaginous callus area), CCA/TCA and more osseous and woven bone are observed in vibration-treatment group according to Leung *et al.* (2005) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat). Similar results are shown by Chung *et al.* (2014) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat), who also describe better bridging and more woven bone at the fracture site in the vibration-treatment group. Shi *et al.* (2010) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat) also describe larger total callus area (Cl.Ar), cartilage area (Cg.Ar) and Cg.Ar/Cl.Ar in the vibration-treatment group, although not statistically significant. Chow *et al.* (2011) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat) report that the vibration-treatment group has the highest mineral apposition rate (MAR) in undecalcified histomorphometry analysis.

Five (Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Usui *et al.*, 1989; Wehrle *et al.*, 2015) of the eight open fracture studies show significantly positive outcomes, while the other three (Bilgin *et al.*, 2017; Uchida *et al.*, 2017; Wehrle *et al.*, 2014) display no positive outcomes in the vibration-treatment groups. Wehrle *et al.* (2015) (LMHFV, 45 Hz, 0.3 ×*g*, open fracture, mouse), Komrakova *et al.* (2013) (HMHFV, 35-90 Hz, 2.5-16.3 ×*g*, open fracture, rat) and Stuermer *et al.* (2010) (HMHFV, 90 Hz, 4 ×*g*, open fracture, rat) report better bridging of the fracture in vibration-treatment groups. Komrakova *et al.* (2013) (HMHFV, 35-90 Hz, 2.5-16.3 ×*g*, open fracture, rat) and Stuermer *et al.* (2014) (HMHFV, 70 Hz. 7.9 ×*g*, open fracture, rat) report a significant improvement of CA and Usui *et al.* (1989) [HMHFV, 25 Hz, two

dimensional, 1.7 ×*g* (horizontal), 2.0 ×*g* (vertical), open fracture, rabbit] show significant increases in callus size and mineralised callus areas. Wehrle *et al.* (2014) (LMHFV, 45/35 Hz, 0.3 ×*g*, open fracture, mouse) do not show any significant differences in bone formation between vibration and control group.

Two studies (Bilgin *et al.*, 2017; Wehrle *et al.*, 2014), using intramedullary pinning and external fixator respectively, present negative results for the histomorphology analysis. Uchida *et al.* (2017) (without fixation) and Shi *et al.* (2010) (K-wire) show a positive trend in the histomorphology analysis, but no significant differences are found. The other nine studies, which report histomorphology assessments, report positive results with significant differences in vibration-treatment groups (Cheung *et al.*, 2012; Chow *et al.*, 2011; Chung *et al.*, 2014; Komrakova *et al.*, 2013; Leung *et al.*, 2009; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Usui *et al.*, 1989; Wehrle *et al.*, 2015).

At week 2 post-fracture (fibrous tissue formation stage), the histomorphology analysis of four studies (Cheung *et al.*, 2012; Chung *et al.*, 2014; Leung *et al.*, 2009; Uchida *et al.*, 2017) find positive outcomes, while Shi *et al.* (2010) show no significant differences between vibration-treatment group and control group. At week 3 post-fracture (hard callus/secondary bone formation stage), two studies (Bilgin *et al.*, 2017; Wehrle *et al.*, 2014) show no positive results in the vibration-treatment group, while Usui *et al.* (1989) and Wehrle *et al.* (2015) report significantly positive outcomes of the vibration treatment. At the bone remodelling stage (after day 21), five studies (Cheung *et al.*, 2012; Chow *et al.*, 2011; Chung *et al.*, 2014; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010) present significantly better results in the vibration-treatment groups while Shi *et al.* (2010) shows a positive trend in vibration-treatment group, but with no statistical significance.

Mechanical testing

Restoration of mechanical properties of fractured bone is a gold standard in animal studies, to reflect complete fracture healing. Thirteen (Butezloff *et al.*, 2015; Gao *et al.*, 2016; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Leung *et al.*, 2009; Shi *et al.*, 2010; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Usui *et al.*, 1989; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014; Wei *et al.*, 2016; Wolf *et al.*, 2001) studies report mechanical testing to evaluate the bone quality, including five closed fracture studies (Butezloff *et al.*, 2015; Gao *et al.*, 2016; Leung *et al.*, 2009; Shi *et al.*, 2010; Wei *et al.*, 2016) and eight open fracture studies (Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Usui *et al.*, 1989; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014; Wolf *et al.*, 2001).

The five closed fracture studies report significantly positive results in the vibration-treatment groups. Ultimate load (UL) and stiffness (N/mm) are significantly higher in vibration-treatment group than in control group, according to Leung *et al.* (2009) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat). Shi *et al.* (2010) report that vibration significantly increases the energy to failure (Web ref. 1) in the vibration-treatment group (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat), while UL and stiffness are increased, but not significantly. According to Butezloff *et al.* (2015) (HMHFV, 60 Hz, 14.4 ×*g*, closed fracture, rat), vibration enhances bone strength (fracture shear load), although no significant differences are found.

Among the eight open fracture studies, five (Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2010; Wehrle *et al.*, 2014; Wolf *et al.*, 2001) show no positive effects of the vibration treatment on fracture healing and three (Stuermer *et al.*, 2014; Usui *et al.*, 1989; Wehrle *et al.*, 2015) report improvement of mechanical parameters in vibration-treatment groups. Among those, one (Wehrle *et al.*, 2015) present significant differences, while the other two (Stuermer *et al.*, 2014; Usui *et al.*, 1989) do not show significant differences. Vibration significantly enhances flexural rigidity in OVX mice, according to Wehrle *et al.* (2015) (LMHFV, 45 Hz, 0.3 ×*g*, open fracture, mouse). Stuermer *et al.* (2014) (WBV, 70 Hz. 7.9 ×*g*, open fracture, rat) show that vibration increases the stiffness and yield load in OVX rats, but with no significant difference. A higher maximum bending moment is observed in vibration-treated groups, by Usui and co-workers [HMHFV, 25 Hz, two dimensional, 1.7 ×*g* (horizontal), 2.0 ×*g* (vertical), open fracture, rabbit], though no significant difference is shown (Usui *et al.*, 1989). Wolf *et al.* (2001) (LMHFV, 20 Hz, 0.03 ×*g*, open fracture, sheep), Stuermer *et al.* (2014) (HMHFV, 90 Hz, 4 ×*g*, open fracture, rat), Komrakova *et al.* (2013) (HMHFV, 35-90 Hz, 2.5-16.3 ×*g*, open fracture, rat) and Wehrle *et al.* (2014) (LMHFV, 45/35 Hz, 0.3 ×*g*, open fracture, mouse) show that the flexural rigidity is not improved by 35 Hz vibration, but it is significantly decreased in the 45 Hz group.

We found that three (Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2010) of the four titanium plate fixation studies and two (Wehrle *et al.*, 2014; Wolf *et al.*, 2001) of the three external fixator studies show negative results for the mechanical testing analysis. Instead, one external fixator study (Wehrle *et al.*, 2015) and a titanium plate fixation study (Stuermer *et al.*, 2014) report significantly positive outcomes for the mechanical testing analysis in vibration-treatment groups. Burezloff *et al.* (2015) (K-wire fixation) and Usui *et al.* (1989) (without fixation) show a positive trend, but no significant difference is found. The other four K-wire studies (Gao *et al.*, 2016; Leung *et al.*, 2009; Shi *et al.*, 2010; Wei *et al.*, 2016) report significantly better outcomes in vibration-treatment groups than in control groups.

At week 2 post-fracture (fibrous tissue formation stage), Butezloff *et al.* (2015) show a positive trend for the mechanical testing in vibration-treatment group, but no significance is found. At week 3 post-fracture (hard callus/secondary bone formation

stage), only Wehrle *et al.* (2015) show significantly positive outcomes for vibration treatment. At the bone remodelling stage (after day 21), four studies (Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2010; Wolf *et al.*, 2001) present negative results, while three studies (Butezloff *et al.*, 2015; Stuermer *et al.*, 2014; Usui *et al.*, 1989) show a positive trend without significant differences. The other four studies (Gao *et al.*, 2016; Leung *et al.*, 2009; Shi *et al.*, 2010; Wei *et al.*, 2016) find significantly better outcomes for mechanical testing in the vibration-treatment groups than in control groups.

Gene expression

Six of the included studies evaluate the gene expression during fracture healing (Chung *et al.*, 2014; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Wehrle *et al.*, 2015). Two of them find that Oc (osteocalcin) gene expression is increased in the vibration-treatment groups (Komrakova *et al.*, 2013; Stuermer *et al.*, 2010). Komrakova *et al.* (2016) show that ALP (alkaline phosphatase) and Oc expression are reduced in the vibration + SR (strontium ranelate) group, compared to SR alone group. However, their 2013 study presents an up-regulation of ALP gene expression

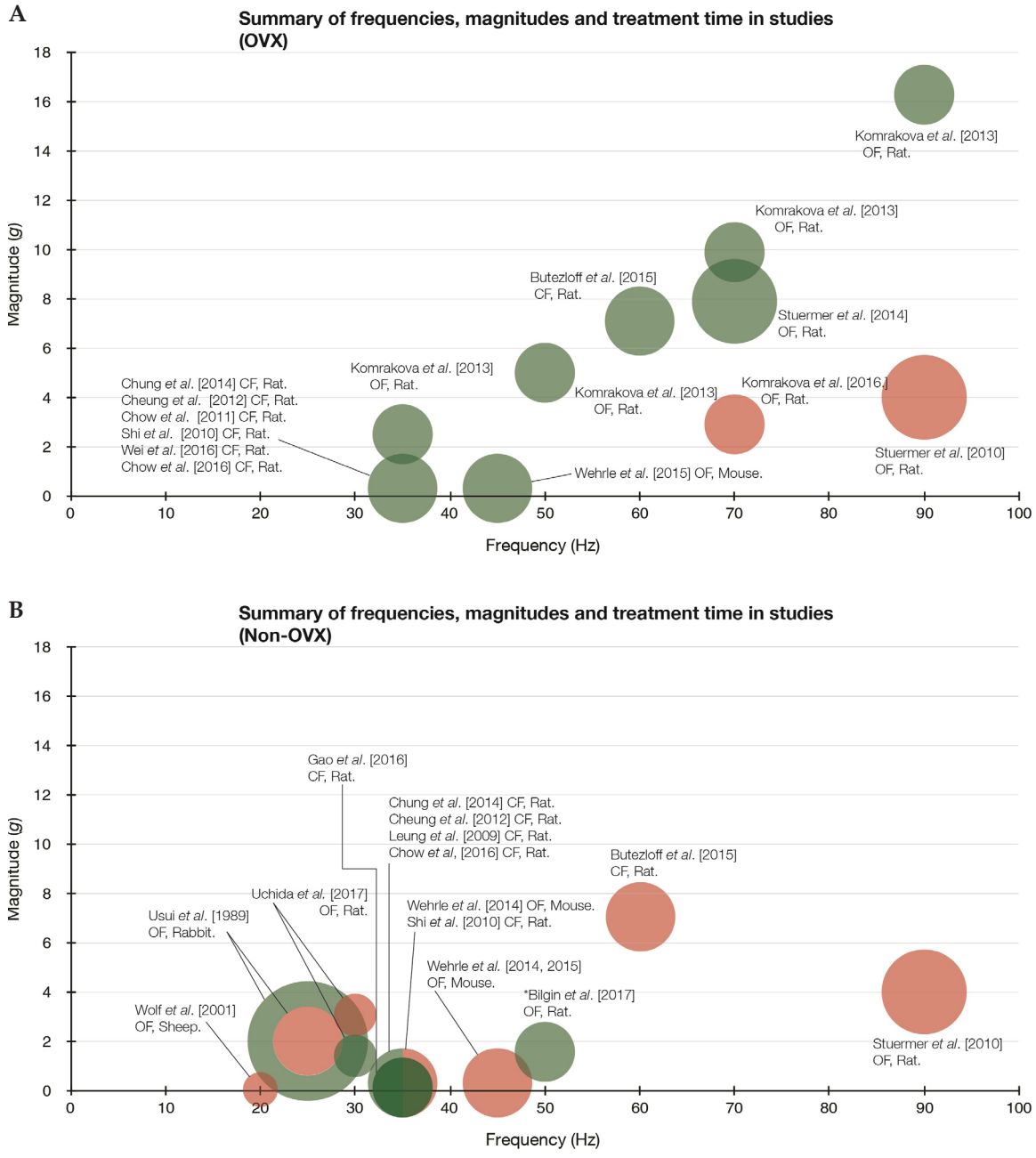

Fig. 2. Bubble chart of frequencies, magnitudes and time of vibration treatment used in studies with (**A**) OVX animals and (**B**) non-OVX animals. Green bubbles indicate studies with positive effects in at least one of the outcome measures; red bubbles indicate studies with no positive or negative effects in at least one of the outcome measures. Area of circle represents treatment duration per day. Only experiments with vertical vibration are included in this figure. OF: open fracture; CF: closed fracture. * Bilgin *et al.* (2017) used male rats in their study.

in the vibration-treatment group (Komrakova *et al.*, 2013). The vibration + PTH (teriparatide) treatment increases OPG (osteoprotegerin) gene expression and OPG/RANKL (receptor activator of nuclear factor κ-B ligand) ratio compared to PTH alone group. Wehrle *et al.* (2015) report that in the non-OVX vibration-treatment group, oestrogen receptor β (ERβ, encoded by Esr2) and Sost (sclerostin) expression are increased, while β-catenin is decreased, as compared to control group. In vibration-OVX group, ERα is up-regulated, compared to non-vibration-OVX group, but ERβ is unaffected. Chung *et al.* (2014) report that vibration up-regulates the expression of collagen type II (Col-2), collagen type I (Col-1), RANKL/OPG. In Stuermer *et al.* (2014), tartrate-resistant acid phosphatase (TRAP) gene expression is significantly down-regulated by E (oestrogen)and R (raloxifeneor) treatment and their combination with vibration.

Oestrogen effect

Two (Stuermer *et al.*, 2014; Wehrle *et al.*, 2015) of the twelve (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Shi *et al.*, 2010; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Wehrle *et al.*, 2015; Wei *et al.*, 2016) studies using ovariectomised animals report the effect of oestrogen supplements on vibration treatment. One study applies ER (oestrogen receptor) antagonisation (ICI) to investigate the roles of ER on fracture healing during vibration treatment (Chow *et al.*, 2016). Nine (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chung *et al.*, 2014; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Shi *et al.*, 2010; Stuermer *et al.*, 2010; Wei *et al.*, 2016) studies use the ovariectomised animals as osteoporosis/osteopenia models to indirectly explore the effects of oestrogen. Of those, five (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chung *et al.*, 2014; Shi *et al.*, 2010; Stuermer *et al.*, 2010) compare between OVX and non-OVX/sham-OVX animals, while four (Chow *et al.*, 2011; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Wei *et al.*, 2016) apply vibration treatment only in OVX animals (Fig. 2).

In the Stuermer *et al.* (2014) (HMHFV, 70 Hz, 7.9 ×*g*, open fracture, rat) and Wehrle *et al.* (2015) (LMHFV, 45 Hz, 0.3 ×*g*, open fracture, mouse) studies vibration treatment alone can enhance mechanical parameters in OVX animals. Stuermer *et al.* (2014) show a positive trend towards higher stiffness and yield-load, even if not significant; Wehrle *et al.* (2015) show that flexural rigidity is significantly increased. Similar results are seen in histomorphology and μCT analyses. Stuermer *et al.* (2014) report that the oestrogen supplement slightly improved the mechanical parameters and the combined treatment of vibration and oestrogen further augment the mechanical parameters in OVX rats. However, according to Wehrle *et al.* (2015) (LMHFV, 45 Hz, 0.3 ×*g*, open fracture, mouse), the increased flexural rigidity and bone formation induced by vibration are abolished by oestrogen supplement in OVX mice. Chow *et al.* (2016) report that the enhanced fracture healing induced by vibration treatment is partially abolished by ICI 182,780 (ER antagonisation).

In the five studies (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chung *et al.*, 2014; Shi *et al.*, 2010; Stuermer *et al.*, 2010), comparing vibration effects in OVX and non-OVX animals, Butezloff *et al.* (2015) (HMHFV, 60 Hz, 14.4 ×*g*, closed fracture, rat), Chung *et al.* (2014) (LMHFV, 45 Hz, 0.3 ×*g*, closed fracture, rat) and Shi *et al.* (2010) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat) show similar outcomes. They report that vibration enhanced fracture healing in OVX animals compared to non-OVX animals, suggesting that osteoporotic/osteopenic bones have a better response to vibration treatment than normal bones. Cheung *et al.* (2012) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat) indicate that vibration can improve fracture healing both in OVX rats and non-OVX rats. In contrast, Stuermer *et al.* (2010) (HMHFV, 90 Hz, 4 ×*g*, open fracture, rat) find that vibration is not effective in both OVX and non-OVX rats, which might be due to an inappropriate regime. The outcomes of two studies by Komrakova *et al.* (2013) (HMHFV, 30-90 Hz, 1.8-16.3 ×*g*, open fracture, rat) and Chow *et al.* (2011) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat), which only applied vibration on OVX rats, show that vibration can enhance fracture healing in OVX rats.

Angiogenesis

Three studies evaluate the alteration of vascular system after vibration treatment (Cheung *et al.*, 2012; Komrakova *et al.*, 2013; Stuermer *et al.*, 2010). Cheung *et al.* (2012) (LMHFV, 35 Hz, 0.3 ×*g*, closed fracture, rat) investigate the effects of LMHFV on angiogenesis and blood flow during fracture healing. They find that vibration enhanced angiogenesis in both non-OVX and OVX rats. Blood flow velocity and vascular volume are significantly higher in vibration-treatment group in both OVX and non-OVX rats. Moreover, vibration improves angiogenesis more in OVX rats, compared to non-OVX rats.

Three other studies explore the effects of vibration on muscle capillaries at fracture sites. Stuermer *et al.* (2010) (HMHFV, 90 Hz, 4 ×*g*, open fracture, rat) show that vibration in OVX rats improves the capillary density in the *longissimus* muscle. Vibration also significantly increases the ratio of capillaries to muscle fibre in OVX rats. Capillary density is slightly higher in the *gastrocnemius* muscle (MG) in both OVX and non-OVX rats, with no significant difference. Also, Komrakova *et al.* (2013) (HMHFV, 35-90 Hz, 2.5-16.3 ×*g*, open fracture, rat) find that vibration (70 Hz, 9.9 ×*g* and 90 Hz, 16.3 ×*g*) significantly enhances the capillary density in the MS (*musculus soleus*) of rats in both OVX and control rats. No significant difference is observed between 70 Hz and 90 Hz vibration regarding vascular density. Komrakova *et al.* (2016) observe that vibration does not change the capillary density in MS. Instead, Cheung *et al.* (2012) observe that vibration alone reduces capillary density in MG in OVX rats, while vibration in the

SR and PTH groups does not change the capillary density. Moreover, VEGF expression is increased after vibration during fracture healing.

Safety

No serious complications or side effects of vibration are reported in any included studies. No body weight or food intake changes are observed in vibration-treatment groups (Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010). No panic behaviour is observed during vibration treatment either (Chow *et al.*, 2011; Leung *et al.*, 2009). Vibration might induce some local complications (inflammations at the pin site), which can be cured within 2 d by H_2O_2 rinsing (Wolf *et al.*, 2001).

Discussion

This paper is the first systematic review on the effects of vibration on fracture healing. We reviewed the current pre-clinical studies on vibration treatment to evaluate its regime, efficacy (in terms of fracture healing and angiogenesis) and safety in normal and oestrogen-deficient animal models. There are, however, no related clinical data.

Suitable mechanical conditions are generally thought to have positive influences on fracture healing (Chao *et al.*, 1998; Claes *et al.*, 1998). Vibration treatment, as a kind of biomechanical stimulation, is proven to have beneficial influences on bone metabolism (Gilsanz *et al.*, 2006; Iwamoto *et al.*, 2005; Ward *et al.*, 2004; Wei *et al.*, 2016). Of all the nineteen studies in this review, fifteen of them (Bilgin *et al.*, 2017; Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Gao *et al.*, 2016; Komrakova *et al.*, 2013; Leung *et al.*, 2009; Shi *et al.*, 2010; Stuermer *et al.*, 2014; Uchida *et al.*, 2017; Usui *et al.*, 1989; Wehrle *et al.*, 2015; Wei *et al.*, 2016) show that vibration has positive effects on fracture healing; two (Stuermer *et al.*, 2010; Wolf *et al.*, 2001) have insufficient evidence to support an improvement of fracture healing after vibration and one (Wehrle *et al.*, 2014) show that 35 Hz vibration does not influence fracture healing significantly, whereas 45 Hz significantly impairs fracture healing. However, 35 Hz vibration still has positive anabolic effects on intact bone. Komrakova *et al.* (2016) find that vibration, together with anti-osteoporosis treatments, can have an adverse effect on bone healing, while vibration alone does not change the bone parameters (Komrakova *et al.*, 2016). Of those fifteen articles, nine (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2011; Chow *et al.*, 2016; Chung *et al.*, 2014; Komrakova *et al.*, 2016; Leung *et al.*, 2009; Shi *et al.*, 2010; Wei *et al.*, 2016) are conducted in closed fracture models and six (Bilgin *et al.*, 2017; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Uchida *et al.*, 2017; Usui *et al.*, 1989; Wehrle *et al.*, 2015) use open fracture models. The distribution of fracture types in the included studies is quite balanced and the type of fracture models seems to have no influence on the effects of vibration. Despite the several studies with no positive or adverse results, vibration treatment can most likely facilitate fracture healing in animals, although only four of them conducted mechanical testing (gold standard to assess fracture healing), showing significantly better mechanical properties in vibration-treatment groups.

Four studies (Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010) use the metaphyseal fracture model with titanium plate as fixation to evaluate the effect of vibration during fracture healing, while the others use the diaphyseal fracture models. In the four metaphyseal fracture studies, none of the mechanical tests show significant differences between vibration-treatment groups and control groups. On the contrary, most of the diaphyseal fracture studies show positive results of vibration. It is generally believed that the healing process of metaphyseal fractures is different from that in diaphyseal fractures. In stable metaphyseal fractures, periosteum ossification with no or limited callus formation is the dominant type of fracture healing. Callus occurs only when the fracture is unstable or there is some displacement (Uhthoff and Rahn, 1981). The dominant healing process in diaphyseal fracture is endochondral ossification. Instead, among the seven studies (Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014; Stuermer *et al.*, 2010; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014; Wolf *et al.*, 2001) using rigid fixation models, only one (Wehrle *et al.*, 2015) shows significantly better results to the mechanical test in the vibration-treatment group. Hence, we speculate that stable fracture with rigid fixation may not be as sensitive to vibration as unstable ones.

Various regimes of vibration treatment for fracture healing are used in the reported studies. Most (12 out of 19) (Cheung *et al.*, 2012; Chow *et al.*, 2011; Chung *et al.*, 2014; Leung *et al.*, 2009; Shi *et al.*, 2010; Wehrle *et al.*, 2015; Wehrle *et al.*, 2014; Wolf *et al.*, 2001) of the selected articles use the low-magnitude high-frequency vibration (LMHFV; < 1 ×*g*, 20-90 Hz), which is reported to prevent bone loss and increase bone mass in postmenopausal women and OVX rats (Iwamoto *et al.*, 2005; Oxlund *et al.*, 2003; Rubin *et al.*, 2004). Komrakova *et al.* (2013) compares the effects of different frequencies and vibration modes on fracture healing, providing vital evidence for choosing appropriate vibration settings. The vertical vibration at 35 Hz (2.5 ×*g*) and 50 Hz (5.0 ×*g*) show better effects on fracture healing in the μCT analysis, histomorphological analysis and mechanical testing. In contrast, the horizontal vibration show no obvious effects on fracture healing. Hence, vertical vibration [35 Hz (2.5 ×*g*) and 50 Hz (5.0 ×*g*)] generated more beneficial effects on fracture healing. Consistent with this study, another report comparing 35 Hz (0.3 ×*g*) and 45 Hz (0.3 ×*g*) reveals that the vibration at 35 Hz gives better results (Wehrle *et al.*, 2014). The flexural rigidity is reduced in the

45 Hz (0.3 ×*g*) group compared to the control group, while the 35 Hz (0.3 ×*g*) group shows no significant decrease of flexural rigidity (Wehrle *et al.*, 2014). Regarding treatment duration, according to Usui *et al.* (1989) [HMHFV, 25 Hz, two dimensional, 1.7 ×*g* (horizontal), 2.0 ×*g* (vertical), open fracture, rabbit], 60 min/d of vibration treatment show better results than 20 min/d. The callus size and mineralised callus area are larger in the 60 min/d vibration-treatment group, indicating that longer duration may induce more benefits on fracture healing. These results match with previous findings according to which longer vibration positively influences bone formation (Judex *et al.*, 2015). This may be a practical aspect to consider for future translation to clinical application when balancing between the beneficial effects and patients' tolerance to the treatment period. To date, no study on vibration magnitude comparison was found in the database. Therefore, more studies on magnitude and treatment duration should be developed to provide more evidence-based regime selection.

Two studies reported that Oc gene expression increases in the vibration-treatment groups (Komrakova *et al.*, 2013; Stuermer *et al.*, 2010). Komrakova *et al.* (2013) also report an up-regulation of ALP gene expression in the horizontal vibration-treatment group. However, ALP and Oc expression are reduced in the vibration + SR group compared to SR alone group. The increase of Oc expression indicates that vibration could promote further bone synthesis at the callus site. The up-regulation of ALP by horizontal vibration (Komrakova *et al.*, 2013) suggests an impaired effect on bone turnover during fracture healing. Vibration down-regulates ALP and Oc expression in SR-treated rats during fracture healing, but the bone parameters does not change, suggesting that ALP and Oc expression at the callus might not correspond to protein synthesis. Vibration + PTH treatment increases OPG gene expression and OPG/RANKL ratio compared to PTH alone group (Komrakova *et al.*, 2013). However, according to Chung *et al.* (2014), RANKL/OPG is up-regulated after vibration + PTH treatment. As reported before, OPG can bind to RANKL and inhibit the differentiation of osteoclasts, which may suppress osteoclastgenesis and enhance osteogenesis in mesenchymal stem cells (MSC) (Boyce and Xing, 2007; Palumbo and Li, 2013). Hence, the increase of OPG and OPG/RANKL ratio reveals that vibration could promote osteogenesis at the fracture site during fracture healing. The conflicting results of OPG/RANKL ratio in these two studies need to be further studied. Wehrle *et al.* (2015) find that, after vibration, ERβ and Sost expression are increased and β-catenin is decreased. ERs could regulate Sost expression, which, in turn, plays a role in inhibiting osteoblast activity. As Wnt signalling *via* β-catenin pathway is identified as a crucial regulator of bone formation and osteoblastogenesis, the decrease of β-catenin might play an important role in fracture healing after vibration treatment (Felber *et al.*, 2015). Chung *et al.* (2014) find that vibration increases the expression of Col-2 and Col-1, which are related to chondrogenesis and osteogenesis, respectively (Kuroda *et al.*, 2005). Stuermer *et al.* (2014) report that TRAP gene expression is significantly down-regulated by E (oestrogen) and R (raloxifeneor) treatment and their combination with vibration. TRAP enzyme encoded by TRAP gene is responsible for bone degradation (Boyle *et al.*, 2003). The decrease of TRAP expression by E, R and their combination with vibration indicates that vibration together with E and R could suppress bone degradation and promote bone formation during fracture healing. Based on the gene expression analysis in the included studies, we also speculated that vibration may improve fracture healing through these gene expressions and their corresponding signalling pathways.

Among the fifteen articles about fracture healing enhanced by vibration, six (Butezloff *et al.*, 2015; Cheung *et al.*, 2012; Chow *et al.*, 2016; Chung *et al.*, 2014; Shi *et al.*, 2010; Wehrle *et al.*, 2015) apply vibration on both OVX and non-OVX animals, four (Chow *et al.*, 2011; Komrakova *et al.*, 2016; Komrakova *et al.*, 2013; Stuermer *et al.*, 2014) only on OVX animals and five (Bilgin *et al.*, 2017; Gao *et al.*, 2016; Leung *et al.*, 2009; Uchida *et al.*, 2017; Usui *et al.*, 1989) are conducted in non-OVX animals. Among the six studies with both OVX and non-OVX animals, three (Butezloff *et al.*, 2015; Shi *et al.*, 2010; Wehrle *et al.*, 2015) show beneficial effects of vibration on fracture healing in OVX animals, while the non-OVX animals show no positive or negative effects. The other three studies (Cheung *et al.*, 2012; Chow *et al.*, 2016; Chung *et al.*, 2014) show positive influence of vibration in both OVX and non-OVX animals during fracture healing (Fig. 2). When comparing Wehrle and colleagues work – one published in 2015 (LMHFV, 45 Hz, 0.3 ×*g*, open fracture, mouse) (Wehrle *et al.*, 2015) and the other published in 2014 (LMHFV, 45-35 Hz, 0.3 ×*g*, open fracture, mouse) (Wehrle *et al.*, 2014) – we noted that these two studies used the same regime but different mouse conditions (OVX and non-OVX) and the outcome were the opposite. Stuermer *et al.* two studies – Stuermer *et al.* (2014) (HMHFV, 70 Hz. 7.9 ×*g*, open fracture, rat) and Stuermer *et al.* (2010) (HMHFV, 90 Hz, 4 ×*g*, open fracture, rat) – also report different results with two different vibration regimes. Hence, we could speculate that oestrogen status and vibration regimes both play important roles in applications of vibration on fracture healing. As it is generally accepted that oestrogen affects cartilage growth and the remodelling process of the fracture callus, the oestrogen status is considered to have influence on fracture healing (Beil *et al.*, 2010). Stuermer *et al.* (2014) (HMHFV, 70 Hz. 7.9 ×*g*, open fracture, rat) conclude that the combination of vibration and oestrogen could improve fracture healing in osteopenic rats. In contrast, Wehrle *et al.* (2015) (LMHFV, 45 Hz, 0.3 ×*g*, open fracture, mouse) find that the enhancement of fracture healing caused by vibration in OVX mice

can be abolished by oestrogen supplementation. Other included studies on OVX animals (Butezloff *et al.*, 2015; Chung *et al.*, 2014; Shi *et al.*, 2010) also conclude that, during fracture healing, OVX animals present better responses to vibration than non-OVX ones. Comparing the vibration settings, Stuermer *et al.* (2014) uses vibration at 70 Hz with an amplitude of 0.4 mm (approximately 7.9 ×*g*), while Wehrle *et al.* (2015) uses a low-magnitude high-frequency vibration at 45 Hz and a 0.3 ×*g* peak-to-peak magnitude. The administration routes of oestrogen are also different: Wehrle *et al.* (2015) implanted subcutaneous oestrogen pellets and Stuermer *et al.* (2014) uses food supplemented with oestrogen, which may explain the discrepancy. Moreover, according to Stuermer *et al.* (2014), BV/TV value detected by μCT and 42 d endosteal callus formation detected by histomorphometry are both decreased in the oestrogen + vibration-treatment group compared to the vibration only group. Even if not significant, in OVX rats, the vital features and mechanical parameters of the oestrogen + vibration-treatment group are found reduced compared to the vibration only group, similar to that reported by Wehrle *et al.* (2015). Oestrogen is found to compete with other signalling molecules for ERα in an osteoblastic model that hinders the strain-activated pathways when ERα is in short supply (Chow *et al.*, 2016; Sunters *et al.*, 2010). Therefore, we postulated that the presence of oestrogen may not be advantageous for fractured bones in response to mechanical loading; instead, based on the evidence, vibration was suggested to enhance osteoporotic/osteopenic fracture healing more than normal bone fracture healing.

Once an intact bone is broken, a haematoma is formed because of the destruction of original vessels. After that, the soft callus is formed with some new blood vessels inside (Carano and Filvaroff, 2003). The new blood vessels are formed through a process known as angiogenesis during fracture healing, which plays a vital role in bone repair. Plantar vibration is reported to increase the peripheral and systemic blood flow (Stewart *et al.*, 2005). Cheung *et al.* (2012) report that VEGF expression is increased after vibration during fracture healing, which is in agreement with the advantageous effects of vibration on fracture healing and may also provide evidence for vibration promoting angiogenesis. Two other studies (Komrakova *et al.*, 2013; Stuermer *et al.*, 2010) on fracture healing also find that the number of capillaries increases in muscles after vibration treatment. On the other hand, Komrakova *et al.* (2016) find no improvement of capillary number in muscles. Although only four of the included studies worked on vascularisation (with three of them having positive results), it can be speculated that vibration can promote angiogenesis in order to enhance bone repair.

The response to vibration is determined by frequency, magnitude, direction, duration of the vibration, acceptability of the participants, body posture and environmental conditions (Griffin, 2012). Most of the studies included in our review suggest that HMHFV and LMHFV are safe in treating fractures. Although there is no safety standard for vibration treatment, the International Standards Organisation provides an occupational health risk evaluation for whole-body vibration (ISO-2631) that can be taken as a reference for vibration safety threshold limit (Griffin, 2012). The regimes of many studies are below the ISO safety threshold and the animals presented no discomfort or complications after the treatment. Based on this evidence, vibration treatment on fracture healing is considered generally safe. As suggested by Muir *et al.* (2013), only those WBV devices, which conform to ISO-2631 guidelines, can be utilised and the selection of vibration platforms for fracture healing should be careful. However, as the study period of vibration treatment in the included studies were short, more long-term studies, both in basic science and clinical trials are essential to validate the safety issue of vibration treatment for fracture healing.

There were several weaknesses in our review. Firstly, as only pre-clinical studies were found, the results should be interpreted carefully. Although they could prove to be of scientific value, this may not be fully clinically validated. Secondly, meta-analysis is not feasible because of the heterogeneity of the included studies, which weakens the conclusion. Moreover, some of the studies were conducted by the same group of researchers, which may lead to a bias. Finally, we only include articles written in English, which probably did not cover all the evidence available.

In conclusion, vibration treatment is suggested to be beneficial in improving fracture healing in animals. However, some studies show insufficient evidence to support an improvement of fracture healing after vibration and one study even demonstrated impairment of fracture healing after vibration treatment. The effects of vibration seem to be highly dependent on the regimes and animal models used, such as age, OVX, *etc.*. Vibration is also well tolerated and generally safe. Oestrogen tends to weaken the effects of vibration during fracture healing. As vibration may develop into a new treatment for fractures, more systematically designed studies are needed to examine the efficacy, regimes and safety for fracture healing. To date, there are no related clinical studies found in MEDLINE. Clinical trials, particularly randomised controlled trials, are therefore essential before translating vibration treatment to clinical applications. The different response between osteoporotic and normal bones to vibration treatment is another key issue to be investigated, which has significant clinical implications to fragility fracture patients.

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Discussion with Reviewer

Volker Alt: How transferable are these data, which are mainly derived from animal data, to the human situation?

Authors: These pre-clinical data should be able to be transferred to human situation because vibrations, with the reported specifications, have already been applied to humans, usually for muscle training or osteoporosis. The vibration settings are proven safe to human, yet clinical trials on fracture healing should be conducted before clinical application.

It should be noted that those animal studies usually used relatively unified fracture type (most are diaphyseal transverse fracture), while clinical cases will be much more complicated, with different fracture types and fixation methods. Therefore, a well-designed large sample size clinical trial including different fracture types is recommended to verify the efficacy of vibration treatment on fracture healing.

Volker Alt: How should an ideal WBV therapy for treatment of patients look like?

Authors: To our knowledge, the first criterion for an ideal WBV therapy for fracture must be safety, as patients with fractures, particularly elderly, are relatively fragile. Also, low-magnitude vibration treatment is recommended for causing less pain to the patients, so that they can comply to the treatment well. Vibration treatment should not introduce any complications, adverse effect or discomfort to the patients. For fracture, vertical vibration is more appropriate than side-alternating one. In general, the vibration platforms meeting ISO-2631 standard are more preferable.

Volker Alt: What clinical data are available on the general effect of WBV on the body?

Authors: Much clinical evidence have been accumulated in the past years on the general effects of WBV on musculoskeletal system. The most convincing data are the effects of vibration on muscle performance, where both, high-magnitude and low-magnitude vibration treatments, are beneficial to muscle activities, including muscle strength, balancing ability, knee-extension strength, postural control, *etc.*. The positive effects on muscle also lead to another benefit of vibration treatment: reducing fall incidences, as proven by several clinical trials. Also, ample evidence supports the effect of vibration on blood circulation, especially in lower limb or plantar. Instead, the effects of vibration on bone is controversial, with a few clinical studies on postmenopausal women indicating positive effects on bone mineral density. However, a few recent clinical trials did not demonstrate any positive effects on bone quality. To date, there are no clinical data on the efficacy of vibration treatment on fracture healing, which instead will be needed in the future.

Editor note: The scientific editor for this paper was Stephen Ferguson.