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REVIEW

Vibration stimuli and the differentiation of musculoskeletal progenitor cells: Review of results *in vitro* and *in vivo*

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Abstract

Due to the increasing burden on healthcare budgets of musculoskeletal system disease and injury, there is a growing need for safe, effective and simple therapies. Conditions such as osteoporosis severely impact on quality of life and result in hundreds of hours of hospital time and resources. There is growing interest in the use of low magnitude, high frequency vibration (LMHFV) to improve bone structure and muscle performance in a variety of different patient groups. The technique has shown promise in a number of different diseases, but is poorly understood in terms of the mechanism of action. Scientific papers concerning both the *in vivo* and *in vitro* use of LMHFV are growing fast, but they cover a wide range of study types, outcomes measured and regimens tested. This paper aims to provide an overview of some effects of LMHFV found during *in vivo* studies. Furthermore we will review research concerning the effects of vibration on the cellular responses, in particular for cells within the musculoskeletal system. This includes both osteogenesis and adipogenesis, as well as the interaction between MSCs and other cell types within bone tissue.

Key words: Mesenchymal stem cells; Mechanobiology; Osteogenesis; Whole body vibration; Adipogenesis, osteoporosis; Low magnitude, high frequency vibration loading

Core tip: Low magnitude, high frequency vibrations are attracting growing interest as a non-invasive therapy for a variety of different disorders. The number of studies aimed at elucidating the effects of vibration both *in vivo* and *in vitro* is increasing rapidly. This review aims to provide an introductory overview of the *in vivo* data for a broad range of human applications and animal models. *In vitro* work is covered in more detail, focusing in particular on studies concerning the effects of vibration on cells derived from the musculoskeletal system.

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INTRODUCTION

Vibration or low-magnitude-high-frequency vibration (LMHFV) loading can be induced clinically by standing a subject on a vibrating platform or *in vivo* by applying LMHFV to cells within culture plates or 3D constructs. It is emerging as a potential stimulus for repair and regeneration of the musculoskeletal system with some preliminary evidence that the mechanism of action is *via* effects on mesenchymal stem cells[1,2]. Clinical trial results are variable, however, as are the results in animal models and cell cultures. Differing loading procedures and parameters make it very difficult to compare between experiments. In this review we aim to provide a series of snapshots of human, animal and cell based trials on the effects on LMHFV loading and related stimuli, with a focus on mesenchymal cells and effects on the musculoskeletal system. This is not intended to be a meta-analysis or a comprehensive review of all studies in this area, but rather to indicate how much further interdisciplinary research needs to be done before we can elucidate the effects of vibration on stem cells.

Within the musculoskeletal (MSK) system, there are a variety of different tissues which work together to allow movement. These include bone, muscle, tendon and ligament, as well as other associated tissues such as blood vessels and nerves. All of these tissues are important in locomotion and posture and are affected by a variety of diseases and by age-related degeneration. It has been well demonstrated and reviewed elsewhere[3-5] that mechanical stimuli are involved in maintaining the structure of these tissues. Too little or too much mechanical stimulation can perturb the cells' mechanobiological signalling pathways, ultimately leading to disruption in tissue structure. In bone, such studies have shown changes in bone formation due to unloading[6], increased osteoblast activity with single periods of dynamic loading[7] or the well-known example of humeral hypertrophy in the playing arm of professional tennis players[8]. Due to an increasing population which is living longer, there is a growing burden on healthcare providers of age related diseases and disorders. In addition to this, many of the aging population are remaining active longer into their lives, putting more stress on their musculoskeletal systems and desiring more effective treatments for injury or degeneration.

One of the major problems for healthcare systems with an aging population is the incidence of osteoporosis, particularly in post-menopausal women. It is estimated to affect one third of women over 50, with changes in oestrogen levels causing a reduction in the bone mass, leading to an increase risk of fractures. In Canada in 2010, it was estimated that $2.3 billion dollars was spent on osteoporosis treatment (1.3% of the total healthcare expenditure). This estimate rises to $3.9 billion when those in long term care with osteoporosis are included. Between 2007 and 2008, Canadian healthcare systems dealt with over 57000 acute care admissions and over 800000 hospitalised days[9]. In Europe, there were 3.79 million osteoporotic fractures and 0.89 million hip fractures in the year 2000. The direct costs of treatment for such fractures is estimated at €31.7 billion and these costs are expected to rise to €76.7 billion by 2050[10]. There is therefore great economic benefit to be derived from improving osteoporosis treatment and care.

There are many other MSK malfunctions which can lead to problems in mobility. Some, such as stroke-induced paralysis, may happen later in life and require suitable rehabilitation methods for older patients. Others, such as cystic fibrosis and osteogenesis imperfecta occur at birth and treatments are required to improve the quality of life in disabled children and adults. Degeneration of the MSK system is also a problem in patients with restricted movement, such as those under long term hospital care. There is a need in all of these situations to improve or maintain the structure and function of the different MSK tissues. It is also desirable in many cases that this is done without the need for an intensive exercise, particularly in those at high risk of fractures or falls or with limited mobility.

In the MSK system, many of the cells which produce and maintain the tissues come from a lineage of progenitor cells contained within the bone marrow. These cells are known as mesenchymal stem cells (MSCs) and similar cells are found in umbilical cord blood[11] and vein[12] and many adult tissues including skin[13] and adipose tissue[14,15]. Therefore, interventions that act on the whole body to improve MSK tissue structure likely have an effect on MSCs as well as mature MSK cells. MSCs are a multipotent stem cell, capable of differentiation along osteogenic, chondrogenic, adipogenic and myogenic lineages[16-18]. Because they are multi- rather than pluripotent some researchers in the field prefer to use terms such as "multipotent stromal cell" or "mesenchymal stromal cell". It is thought that MSCs could be a potent tool for regenerative medicine, allowing the repair of many tissue types within the body. Regenerative medicine aims to develop methods to recruit or guide the MSC pool of cells *in vivo*, enabling them to more effectively augment healing. The related field of tissue engineering is a repair strategy in which stem cells are stimulated to differentiate towards a required lineage, usually *in vitro* and then implanted into a patient. Expansion of a patient's own cells may necessary for such therapies, due to the relatively low frequency with which these cells are encountered within the bone marrow (0.01%-0.001%)[19,20]. These cells are also known to be immunomodulatory, reducing strong

inflammatory responses within tissues[21-23]. There is some hope that allogeneic MSCs may be of use during therapy, allowing for a more immediate source of cells than patient expanded MSCs.

In vitro, the most commonly used methods to induce differentiation of MSCs involve glucocorticoids such as dexamethasone[24], growth factors such as bone morphogenic proteins (BMPs)[25,26] and other biochemicals such as ascorbic acid[27] and β-glycerophosphate[28] (for the deposition and mineralisation of bone matrix). For a general review of such methods, see Delaine-Smith and Reilly (2011)[29]. For transplantation of stem cells or their use in tissue engineering, it may be possible to use chemical means to direct differentiation. For *in vivo* applications, the use of chemical agents will have other systemic effects and the cost of growth factors is prohibitive for most health services. Therefore non-biochemical means of stimulating MSCs could be very useful.

There is growing evidence that mechanical stimuli can be used to direct stem cell differentiation towards a variety of different tissue lineages, reviewed elsewhere[30-33]. For example, in MSCs it has been demonstrated that the application of compression[34], tension[35] or bending of scaffolds[36] can increase osteogenic differentiation. Other work has shown that microgravity can reduce the osteoblastic differentiation of human MSCS[37]. *In vitro* work on such stimulation techniques may allow the development of methods to enhance recruitment and differentiation of such cells *in vivo* and allow more targeted therapies. The principle behind LMHF loading is that such vibrations are similar in to the tiny, short duration forces which are applied to bones through muscle action in resting. There is evidence that these small motions may be more important than high impact events in maintaining bone mass and aiding repair[38].

The following sections of this review will cover the *in vivo* and *in vitro* use of LMHFV. The range of measures for *in vivo* treatments will be covered briefly, with a more in depth look at studies concerning cell behaviour and progenitor recruitment or differentiation in the MSK system. The final *in vitro* section will include studies on differentiated cells before continuing to consider the growing body of literature on progenitor differentiation.

WHOLE BODY VIBRATION - LMHFV *IN VIVO*

In the treatment of bone breakages, there is a need to ensure that appropriate mechanical cues are provided to the tissue in order that it is properly maintained and able to regenerate. Fixation devices, both internal and external, may be used to maintain the position of the broken bones in order to facilitate healing. Such devices can reduce the need to avoid weight-bearing on the limb, preventing disuse which is detrimental to the bone tissue. There are also defects that will not heal with fixation alone, leading to non-union fractures. Where standard treatments have proved ineffective in repairing the bone tissue, there is a need to find alternative therapies for such patients. In addition, people with osteoporosis are at high risk of fracture and the best method of fracture prevention in this group is to maintain bone mass.

These clinical problems have led to the investigation of methods of mechanical stimulation to improve bone mass and healing, including LMHFV, applied by having the subject stand on a vibrating platform[39-41]. The acceleration used during these experiments is usually very low (less than the acceleration due to gravity) and the magnitude is typically less than 1 mm. The frequency varies between studies, commonly ranging from 10 to 150 Hz. In addition to different inputs, a variety of different outcomes have been measured. These include bone mineral density (BMD), trabecular width or trabecular spacing and bone formation rate (BFR), but also include measures of balance[42], likelihood of falls[43] and jump height[44]. In addition, other groups have considered the effects on MSC populations, such as number, location and differentiation capability[1,45].

There are many *in vivo* studies which have looked at the amount and quality of bone after LMHFV stimulation. Large and small animal models have been used, as well as clinical human studies. In such clinical studies, LMHFV is often referred to as "whole body vibration"[43,46-49]. Such studies have covered healthy patients or those with compromised skeletal systems, in particular post-menopausal women. The efficacy in treating other conditions, such as recovery after stroke[50], improving the quality of life for disabled children (see Matute-Ilorente *et al*[51] for a review on this topic) or limb function in patients with chronic obstructive pulmonary disease[52] have also attracted interest. Some studies have also included the ability of LMHFV to improve performance and training in athletes[53] and cognitive performance in adults with or without attention deficit hyperactivity disorder[47]. Studies have also used microvibration (20 Hz rocking platform) to improve the implantation rates of embryos undergoing IVF treatment[54].

In the case of *in vivo* murine studies, animals are usually confined in a box or cage which can be vibrated in order to apply the LMHFV stimulation[1,55-59]. It is worth noting that in such experiments the animals are able to move about freely during the stimulation period. As human participants are usually asked to stand still on a vibrating platform, this may explain some differences in results between the two species. Compliance in animal trials is assured as their whole environment is often vibrated, but is often low with human patients and may affect the potency of the treatment. The normal movement of the animals during the stimulation, such as running, resting or standing on two legs, will affect the transmissibility of the vibrations through the body, further altering the response to loading. The breadth of

studies reported in the literature cover different vibration conditions, with differing frequencies and accelerations (often provided as a multiple of the acceleration due to gravity, *g*). Ranges include 0.3-1.5*g* and up to 90 Hz, applied over different timescales, in mice ranging from juvenile to old. With murine studies, researchers often consider several sites of interest (several bones for example) due to the ease of analysis after sacrifice. mRNA levels of markers of osteogenic activity are often measured, as well as the density of bone in different regions.

In young healthy patients, a study by Torvinen *et al*[44] noted an increase in jump height for patients after 8 mo of LMHFV treatment [25-40 Hz (2-8*g*), 4 min/d, 3-5 d/wk]. This was not accompanied by any changes to bone mass, structure or turnover however. In studies of post-menopausal (PM) women, reported results include small increases in bone mineral density with high compliance (0.2*g*, 30 Hz, up to 20 min per day, every day for one year)[40], slight increase in BMD and no change in bone markers (0.2*g*, 30 Hz, 2/d for one year)[39] or improvements in femoral neck BMD and balance of the participants (12.6 Hz, 3 cm amplitude, 6 min x 1 min bouts, 3 sessions per week for 8 wk)[42]. In another study combining exercise and LMHFV, a reduction in the number of falls in PM women was noted[43]. The study included 15 min of LMHFV during a 60 min exercise program and also found no significant differences in the BMD of the spine or hip in the subjects. Such studies suggest that the LMHFV may be having effects on other parts of the musculoskeletal system to improve a variety of measured outcomes. LMHFV has also been shown to cause a small improvement in muscle power in PM women in a short term study, despite low compliance[60]. Such studies vary in terms of length of treatment, outcomes measured and conditions applied however, creating many difficulties in analysing the usefulness of LMHFV. These differences are often compounded by low compliance and small study size, making it difficult to draw conclusions about the efficiency of LMHFV on the MSK system.

DISUSE AND DISABILITY

Vibration has been shown to be effective in the prevention of bone loss due to bed rest. Healthy students were subjected to 56 d of bed rest with or without treatment of vibration combined with resistive exercise[46]. Vibration treatment was found to prevent the changes in muscles size and function, as well as bone volume, compared to the start of the study. Bone mineral content was also reduced in the control subjects but not those treated.

Muir *et al*[61] studied the effects of 30 Hz LMHFV (10 min per day, 0.3 and 0.5*g)* during periods of bed rest and found that subjects retained muscle flexion strength but not extension strength. Subjects were also found to have better postural stability compared to controls. Miokovic *et al*[62] looked at vibration combined with resistive exercise over 60 d bed rest, finding slightly improved pain scores in the vibrated compared to exercise along groups but no other significant differences. Holguin *et al*[63] found that LMHFV (30 Hz, 10 min per day) was able to reduce swelling in the intervertebral disc of human volunteers due to 90 d of bed rest, as well as reducing the reported incidence of back pain by 46%. Other work by the authors has shown that shown that LMHFV (90 Hz, 0.2*g*, 15 min/d for 4 wk) can preserve spinal disc mechanics during periods of unloading[64]. This work was carried out using an animal model however, other examples of which are discussed later in this review.

From the current body of clinical data, LMHFV shows promise in a variety of disease states to improve tissue structure or quality of life. There are fewer studies showing beneficial effects of LMHFV in healthy patients, suggesting that LMHFV may be not be suitable for improving already healthy tissue.

TRANSMISSIBILITY

An important factor in the use of whole body vibration as LMHFV is the transmissibility of the vibrations through the rest of the skeleton. Work in human subjects by Rubin *et al*[65] studied the transmissibility of LMHFV through the spine and hip (15-35 Hz, 36 N). They found that for frequencies lower than 20 Hz, there was low transmission of the forces through the body. However, resonances of the vibrations occurred at higher frequencies, which may help explain why higher frequencies appear to be more effective. Kiiski *et al*[66] also investigated transmissibility of LMHFV, ranging from 0.05-3 mm amplitude and 10-90 Hz. They found amplification of the peak amplitude at low frequencies at the ankle, knee, hip and spine. At the spine, this occurred only for 10 Hz acceleration, rising to include 40 Hz by the ankle. Above 40 Hz, transmissibility was reduced 10 to 1000-fold. All subjects reported discomfort for vibration between 20 and 25 Hz for displacements larger than 0.5 mm (peak acceleration of 0.8-7.5*g*), with one suffering numbness in the feet at 40 Hz (3 mm amplitude, 19*g*). These observations suggest that high acceleration vibrations may not be suitable for LMHFV therapy as patients may be less likely to comply if the treatment was uncomfortable. The effects of these high acceleration conditions over the longer term are not well studied and it is not known whether this discomfort will translate to negative effects on the MSK system. The trial had a very small number of participants however and the use of external accelerometers may have affected the results. A recent review of the literature concerning osteoporosis treatments[67] included a section on LMHFV, recommending parameters of 0.3*g*, 25-45 Hz, as most positive studies fall within this range of frequencies stated, and upright posture. There were also several recommendations regarding contraindications, including cancer, severe diabetes and

recent surgery or implantation. The authors suggest that lower frequencies may be unsuitable as they can cause vibration in the internal organs and notes that accelerations higher than 1*g* have been observed to cause side effects such as back pain.

One study in disabled children used short bouts of LMHFV treatment (0.3*g*, 90 Hz, 10 min/d) 5 d per week over 6 mo and found increases in BMD in tibial and spinal regions compared to a placebo device[68]. There were however no observed changes in diaphyseal bone or muscle parameters. The study noted problems with compliance and stance of the children during the vibration and speculated that variation in posture of the children during vibration therapy may have been one factor in the relatively minor benefits seen. This suggests that transmissibility is an important and unresolved issue for the successful application of LMHFV for therapeutic use.

ANIMAL MODELS AND LMHFV

Much as there have been a range of conditions tested in human trials of LMHFV, there are a broad spread of *in vivo* animal studies. These cover the use of large animal models in sheep to small animal models in rats and mice. Some studies use healthy animals while others have included ovariectomised animals to emulate post-menopausal conditions. Outcome measures and study lengths also vary greatly and this next section provides an overview of the animal models used so far with LMHFV studies.

In a large animal model of LMHFV, the application of vibration to the hind legs of sheep (30 Hz, 0.3*g*, 20 min/d) 5 d a week for one year showed improvements in the bone structure[69]. Bone mineral content and trabecular number were increased, as well as the longitudinal stiffness and strength of the tissue. The trabecular spacing was seen to decrease, demonstrating bone adaptation and strengthening. Following on from this work, Judex *et al*[70] looked at the same vibration conditions and modelled the trabecular stiffness in different directions from the femoral condoyle. The models predicted increases in the stiffness in longitudinal, anterior-posterior and medial-lateral directions through the tissue and more uniform distribution of off-axis loading. They also noted that the bone volume and connectedness of the tissue were increased in the vibrated group, demonstrating adaptations requiring bone remodelling.

OSTEOPOROSIS MODELS AND AGING MICE

As LMHFV offers a simple, non-invasive treatment with the potential to improve bone mass and structure, there many studies investigating its use for the treatment of osteoporosis. Therefore there are a large number of animal studies attempting to clarify the possible benefits of vibration by using Ovariectomised (OVX) or aged rodents.

OVX rats or mice are a commonly used animal model of osteoporosis, where the animals develop osteoporosis-like symptoms (such as reduced BMD and decrease in trabecular number) in the months after ovariectomy[71]. Work by Flieger *et al*[72] followed OVX rats for 5 or 12 wk post-surgery, some of which were treated with LMHFV 5 d per week (50 Hz, 2*g*, 30 min/d). Significant increases in BMD were found in the femur and tibia (vibrated compared to non-vibrated), which gave a non-significant increase in the fracture load of the femur. The strength of the femur, however, was lower for vibrated animals than for any of the SHAM groups, although there was a non-significant increase for OVX combined with vibration compared to OVX only. Oxlund *et al*[73] found that vibration was able to prevent the loss of bending and compressive strength seen in OVX rats. Vibration (0.5*g* 17 Hx, 1.5*g* 30 Hz, 3.0*g* 45 Hz) given for 30 min per day over 90 d prevented endocortical resorption and increased bone formation. Although an increase in BFR was also seen in the OVX only animals, the increase was greatest for the 45 Hz vibration condition.

A later study also demonstrated the prevention of the detrimental effects of ovariectomy by LMHFV[58]. 3-mo-old rats underwent ovariectomy (OVX) or a sham surgical procedure (SHAM), after which they were left untreated for 3 mo such that the OVX group developed osteoporosis. At this time, half of the animals from the OVX and SHAM treatment groups received LMHFV therapy at 90 Hz (2 × 15 min/d, 7 d/wk for 35 d). Yield load and Young's modulus of the fourth lumbar vertical body were restored to the level of SHAM animals. These changes were accompanied by increases in density (particularly for lumbar trabecular bone). Increases were also found in trabecular bone area, trabecular number and width and both the percentage of cortical bone and BMD. The vibration stimulus was found to be anabolic regardless of the oestrogen levels in the animals.

LMHFV has also been tested to try and reduce the effects of secondary osteoporosis caused by glucocorticoid treatment[74]. Three-month-old mice were placed into three groups-control, glucocorticoid treatment and glucocorticoid treatment with LMHFV. Five days per week for 9 wk animals were given saline (control group) or methylprednisolone (glucocorticoid groups) injections. Vibrations were applied at 60 Hz during the same period (1*g*, 30 min per day, 5 d per week for 9 wk). Glucocorticoids alone reduced weight gain, tibial bone mineral content (BMC) and trabecular number and increased trabecular spacing compared to controls. The animals given vibration therapy showed no difference in BMC compared to controls but higher trabecular number and lower spacing than the glucocorticoid only group. This suggests that LMHFV may be a suitable therapy for limiting drug induced

changes in bone quality, although further studies on this topic are necessary.

A longer term study by Wenger *et al*[59] in elderly mice (18-mo-old) found no change in the bone volume or strength but increased mineralisation in vibrated animals (0.5 and 1.5*g*, 32 Hz, 30 min/d for 12 wk). Mineralising surface was calculated histologically and the research examined the femur, radius and lumbar vertebrae of the animals. No changes due to vibration were seen in the lumbar spine, however in the femur there was an increase in high density bone. Vibration also reduced the number of pyridinoline crosslinks at both accelerations, suggesting a reduction in the breakdown of collagen. High density bone volume was increased at 0.5*g*, with an increase in osteoclast numbers at the higher acceleration. Both vibration conditions showed significant increases in mineralisation surface compared to controls. There was however no significant difference in failure load or stiffness of the radius between any vibration or control condition. This demonstrates that not only are the correct conditions necessary to have an effect on bone turnover, but slight changes to a regimen may be enough to alter the outcome.

Other studies have considered the site specificity of the responses to LMHFV[75]. They used 0.7*g* LMHFV at a variety of different frequencies applied for 4 wk (10 min per day, 5 d per week) and studied several skeletal sites in mature male mice. They found that the low frequency used (8 Hz) increased resorption and reduced mineral apposition, weakening the skeleton. 90 Hz vibrations increased trabecular reorganisation and mineral apposition without affecting resorption. These effects occurred for different magnitudes in the vertebrae, tibia and femur, demonstrating the variable effects of LMHFV through the MSK system. The results also demonstrate that, under the wrong conditions, LMHFV may have negative effects on bone tissue.

Judex *et al*[76] studied vibration (45 Hz, 0.3*g*) for 10 min per day (5 d/wk, 21 d) compared to hindlimb unloading in a murine model. They found increased bone formation and mineralisation in the trabecular and periosteal regions of the vibrated tibia. After 4 d in the disuse condition, there were lower levels of mRNA expression for several markers of osteogenesis (col-I, osteonectin, osterix and MMP-2) but no changes were seen for the vibrated group. At 21 d, significant increases were seen for nitric oxide synthase, MMP-2 and RANKL in the vibrated group. The work suggests that short periods of vibration may be able to prevent the bone loss and reduction in osteogenesis which occurs during disuse. As has been seen in human studies, the best results in animal models are obtained in subjects with a lower than normal BMD. Animal models often show better results than human studies, which may be due to guaranteed compliance in the animals and lower rates in human cohorts. There are still many unanswered questions, particularly regarding the efficacy of higher acceleration vibration stimuli.

OSTEOGENESIS IMPERFECTA

Osteogenesis imperfecta (OI), in which the structural protein type 1 collagen is mutated, is another condition which may benefit from LMHFV intervention to improve bone mass and structure. The "brittle bones" which characterise the condition can cause limited to severe changes in stature, skeletal deformities and moderate to severe bone fragility[77]. The condition is difficult to treat and while bisphosphonate drugs can be used to inhibit resorption, these affect the normal bone remodelling process and can prevent maintenance of healthy tissue. In a young mouse genetic model of OI, LMHFV (0.3*g*, 45 Hz) was applied 5 d a week for 5 wk, starting when the mice were 3-wk-old[57]. They found that cortical bone thickness and area was increased for both OI and wild type mice in the tibia and femur, whereas trabecular bone volume was increased for the vibrated mice in the tibia. These changes were accompanied with an increase in femoral stiffness and yield load. They noted that there were only minor differences in bone apposition between the vibrated and sham groups, suggesting that the vibration reduced bone resorption.

FRACTURE HEALING

Several groups have studied the effects of LMHFV on the healing of fractures in murine models. Leung *et al*[78] used LMHFV at 35 Hz (0.3*g*, 20 min per day, 5 d per week) beginning 5 d after the creation of femoral fractures in 3 mo old rats. They found that a larger callus was formed by 2 wk of treatment and increased remodelling had occurred after 4 wk in the vibrated animals compared to those in a sham treatment group. The mechanical strength of the femur was also higher in the LMHFV group, suggesting that the treatment was able to enhance the fracture healing by altering accelerating callus formation and turnover.

Later work using the same vibration conditions studied the effects of LMHFV on fracture healing in rats treated with ibandronate (a bisphosphonate) to suppress bone remodelling[79]. In this study, 6 mo old rats underwent ovariectomy and were left for 3 mo to develop osteoporosis. Rats then underwent surgery to create closed femoral fractures before receiving ibandronate treatment (with or without LMHFV), LMHFV alone or sham treatment. The vibration only group showed improved speed of callus reduction, increased mineral apposition and increased serum markers of bone turnover compared to all other treatment groups. This remodelling was disrupted by the bisphosphonate treatment, but the application of LMHFV during bisphosphonate treatment was able to reduce the delay in bone remodelling caused by the drug. This provides further evidence that LMHFV may be useful in mitigating the effects of bisphosphonate treatment by reducing the negative effects on bone remodelling.

In an OVX rat model, Shi *et al*[80] used LMHFV to

augment fracture healing in OVX rats compared to SHAM control animals. Animals were treated with LMHFV (35 Hz, 0.3*g*, 20 min/d) for 5 d per week up to 8 wk. In OVX animals, callus formation and mineralisation, as well as remodelling activity and energy to failure were reduced compared to the SHAM animals. LMHFV was found to improve fracture healing, with OVX + vib showing improved fracture healing compared to SHAM + vib. This suggests that the mechanical stimulus is more effective in animals with reduced oestrogen levels, but is anabolic regardless, similar to the findings by Sehmisch *et al*[58]. The effects of LMHFV on fracture healing in osteoporotic and normal bone have also been investigated in terms of angiogenesis[81]. Vibration treatment improved blood flow and angiogenesis for both sham and OVX animals, and overall improved fracture healing.

Work at a higher vibration frequency (90 Hz, 4*g*, 15 min twice per day, 30 d) demonstrated some beneficial effects of LMHFV on fracture healing in OVX rats[82]. Three months old rats underwent ovariectomy and showed severe osteopenia 10 wk post-surgery. At this point, both OVX and untreated rats underwent bilateral metaphyseal osteotomy of the tibia, before beginning LMHFV or sham treatment 5 d later. After 30 d of LMHFV or sham treatment, the group found that LMHFV had improved bridging of the fracture in the OVX but not the intact rats, as well as improving the densities of cortical bone and callus. They found a reduction in the stiffness and yield load of the tibia after vibration and suggest that this may be due to the high acceleration used for the LMHFV treatment, which is similar to the result seen by Flieger *et al*[72]. These studies suggest that it may be possible to use LMHFV to improve fracture healing, whilst reinforcing the idea that the conditions must be carefully tailored in order to prevent detrimental effects of LMHFV.

TENDON AND MUSCLE

Work investigating the effects of LMHFV on tendon structure has shown an increase in cross-sectional area, stiffness and strain to ultimate load. Sandhu *et al*[83] used a rat model and 5 wk of LMHFV (0.3*g*, 30 Hz, 5 d/wk) to study the flexor carpi ulnaris tendon. Their results suggest that LMHFV may also affect tendon tissue within the MSK system which may be useful for tendon healing if damage has occurred to a joint.

LMHFV has also been shown to affect muscle tissue. In skeletally immature mice, LMHFV was found to affect muscle structure in the soleus muscle[56]. After 6 wk of stimulation for 15 min per day (0.3*g*, 45 Hz), the number of arterioles, venules and capilliaries within the muscle fibre were reduced. This was significant in the end regions of the muscle. Although a reduction in vasculature to the muscle tissue may appear to be an undesirable adaptation to LMHFV, a study by Xie *et al*[84] under the same conditions showed increases in the moment of inertia and area of the soleus muscle. The same study also found increases in trabecular and periosteal bone volume, bone marrow and cortical bone area but no change in osteoclast activity. These studies provide early evidence that LMHFV is capable of effecting different parts of the MSK system and may be able to aid regeneration in complex injuries to multiple tissues.

The variety of animal studies considered above demonstrate the range of interest in LMHFV as a therapy to affect the musculoskeletal system. These studies have been conducted in small, quadruped animals and as such may not be directly applicable to human conditions. Many of the studies also use 0.3*g* acceleration or 45 Hz, with results suggesting that these conditions in particular may provide beneficial effects. This matches with the recommendations of Katsuri for the use of LMHFV to treat osteoporosis in humans[67]. The research does suggest that the technique is able to affect many different cell types to alter the properties of a variety of tissues including bone, tendon and muscle. In the case of bone, evidence suggests that LMHFV may be able to improve poor bone structure or aid in healing. If the conditions used in these studies are translated directly into human trials, such as in the work by Luu *et al*[85] discussed in the next section, it may help develop the understanding of LMHFV as a therapy.

LMHFV AND CELLS *IN VIVO*

The effects of LMHFV on cells *in vivo* have also been investigated in a range of studies, often in combination with measures of bone properties. Over 5 wk of vibration, Christiansen and Silva[1] found several site specific responses to loading for a range of stimulation conditions. Adult mice (7-mo-old) were vibrated with accelerations of 0.1, 0.3 and 1*g* at 45 Hz (15 min/d, 7 d/wk) for 5 wk before sacrifice. Bone marrow was extracted and tibiae, femurs and L5 vertebrae scanned using micro CT. The 0.3*g* vibration condition showed no differences in bone volume over total volume compared to controls at any site, but was significantly lower than other vibration conditions in the L5 vertebra and proximal tibial metaphysis. Researchers noted that the distance from the vibration platform did not dictate the efficacy, nor was the response seen dose dependant. Although there was a decrease in the presence of progenitor cells, no increase in bone formation was seen. The ALP activity of adherent BMSCs, extracted from the bone marrow of these animals and cultured for 2 wk was not significantly different between any groups, however alizarin red staining showed a reduction in mineralisation for 0.1*g* (compared to 1.0*g*) and 0.3*g* (compared to control and 1.0*g* samples).

Work in mice has demonstrated that LMHFV may be used to inhibit adipogeneis, with 7 wk old mice undergoing a 15 wk treatment protocol[86]. They applied LMHFV at 90 Hz (0.2*g*, 15 min per day, 5 d

per week) and found lower volumes of fat (normalised to body mass) for the vibrated compared to sham control groups. Their fat also contained lower levels of triglycerides, indicating a lower risk for the development of diabetes. The work also looked at the differentiation of adipogenic precursors in a separate experiment. Eight weeks old mice were irradiated with 15 kGy of gamma irradiation to kill the bone marrow before receiving an injection of MSCs from donor mice. The donor mice express green fluorescent protein in all their tissues, allowing the donor cells to be monitored *in vivo*. Mice were allowed one week to recover before receiving LMHFV or sham treatment for 6 wk. The group found that the vibrated mice had a lower ration of GFP expressing adipocytes to MSCs compared to controls, as well as reduced weights of the epididymal fat pad, suggesting the MSCs were less disposed to adipogenic differentiation.

Work by Luu *et al*[85] demonstrated the effects of LMHFV on the osteogenic and adipogenic differentiation of MSCs in a diet-induced obesity mouse model. They also applied LMHFV at 90 Hz (0.2*g*, 15 min per day, 5 d/wk) to 7-wk-old mice, in this case for 12 wk and compared this to a sham control with no vibration. Twelve mice received a high fat diet only during the vibration period (prevention of dietary obesity), while another 8 started the diet at 4 wk of age (reversal of obesity). In the prevention group, mice treated with LMHFV for 6 wk showed increased MSC numbers compared to controls, upregulated transcription of Runx2 and downregulation of peroxisome proliferator-activated receptor γ (PPARγ), suggesting a more osteogenic lineage with reduced adipogenic behaviour. After 12 wk of treatment this translated to increased bone volume fraction and reduced weight gain compared to the controls. However, in the mice starting the high fat diet at 4 wk of age, there were no differences in adiposity or bone volume between control and vibrated animals, suggesting LMHFV is not able to reduce existing obesity.

Interestingly, the group translated this work into a human trial in young women with low BMD, reported in the same study[85]. After 12 mo treatment they found an increase in BMD in the spine and no significant change in visceral fat area compared to the start of the study in LMHVF treated patients. In the control group, there was no change in BMD and an increase in fat, suggesting that the technique is also able to increase osteogenesis and reduce adipogenesis in humans. Both this work and that by Rubin *et al*[86] suggest that LMHFV is able to inhibit adipogenesis in MSCs as well as increasing osteogenesis. This reduction in adipogenesis may be a beneficial side effects of LMHFV treatment, but the research also suggests it will not be suitable as a method to reduce pre-existing obesity.

In a hindlimb unloading murine model, Ozcivici *et al*[45] compared 15 min of weight bearing each day (HU animals) to the same period of vibration (HU + vib, 0.2*g*, 90 Hz). This was carried out for 3 wk, at which point animals were sacrificed or underwent a further 3 wk period of reambulation, continuing with the same sham (RA) or vibration (RA + vib) treatments. For animals sacrificed prior to reambulation, the study found no difference between sham and HU + vib conditions for trabecular bone fraction. However, the animals which had received the vibration stimulus showed several changes at a cellular level. The osteogenic MSC and osteoblast populations were found to be greater and HU + vib animals also showed a smaller osteoclast surface. Reambulation magnified these differences, with a greater increase in bone volume, MSC population, osteoblast surfaces, BFR and a higher ratio of bone-lining osteoblasts to marrow adipocytes (RA + vib compared to RA). They conclude that, although vibration does not prevent the bone loss due to disuse, there are improvements in cellular function which may enhance long term recovery. The work suggests that the overall effects on bone fraction may be due to an effect of LMHFV on MSCs within the bone marrow.

A recent study investigated the use of LMHFV to accelerate wound healing in a diabetic mouse model[55]. 30 min of LMHFV (0.4*g*, 45 Hz) was applied for 7 or 14 d after wounding of the skin and compared to sham loading controls. There was an increase in angiogenesis and granulation tissue formation seen at day 7 in vibrated animals. By day 14, this had led to accelerated wound closure and re-epithelialisation. The study monitored levels of growth factors and cytokines associated with promoting healing, with increases in the vibrated group for IGF-1, VEGF, monocytes chemotactic protein-1. Additionally, while there was no effect of vibration on $CD11b^+$ cells in the wounds, $CD11b^-$ cells displayed a less inflammatory phenotype.

In a model of granulosa cell tumours in mice, LMHFV has been shown to help mitigate the associated osteopenia[2]. LMHFV (0.3*g*, 90 Hz, 15 min bouts) was applied 5 d per week over the course of a year and found no significant changes in survival rates of the animals. They found increases in the bone volume in vibrated mice, as well as reductions in tumour incidence and the number of organ systems involved. The research also found that the number of MSCs in the bone marrow were significantly lower in vibrated mice and those lacking pathology, suggesting that they may play a role in the disease progression.

All of these studies suggest that LMHFV is having effects on a variety of cell types *in vivo*, particularly the proliferation and differentiation of MSCs. Possible benefits include increasing osteogenesis and reduction of adipogenesis. The studies suggest that higher frequency LMHFV (90 Hz) at a lower acceleration (0.2*g*) may be able to stimulate progenitor cells within the bone marrow. However, more studies at 0.3*g* covering the effects on MSCs *in vivo* will help to clarify the effects.

LMHFV AND *IN VITRO* CELL STUDIES

In order to better understand the mechanisms of

responses to LMHFV and to identify which cells types are involved, many *in vitro* studies have been undertaken. For bone, there are several possible responsive cell types including osteoblasts, osteoclasts or their progenitors. As a variety of other effects have been seen *in vivo*, such as those on muscle and tendon, there may be further cell types which are responsive to LMHFV. *In vivo* stimulation of tissue forming cells also has applications in the field of tissue engineering where mechanical forces have been well demonstrated to modulate tissue formation by cells, but it is difficult to design bioreactors and stimulation regimens that could be scaled up for clinical and commercial use. LMHVF stimulation may be an appropriate method for the application of mechanical forces to variety of scaffold types and complex geometries, as well as allowing the stimulation of multiple samples within standard culture apparatus.

Experiments conducted *in vitro* include the use of different cell types as well as vibration parameters (frequency, amplitude, duration, number of applications, timeframe). One cell type often used to study osteogenic differentiation with LMHFV is the MC3T3-E1 cell line, a murine osteoblast precursor line. A variety of different outcomes have also been measured, including mRNA expression of different molecules, matrix expression and deposition and prostaglandin E_2 (PGE_2) release.

Dumas *et al*[87] looked at the effects of LMHFV on MC3T3-E1 cells and also the ability of the ECM they deposited to affect MSC attachment and differentiation. The cells were stimulated with 15-40 microstrain (measured as deformation of the cell culture dish) at 400 Hz (20 min per day for 1, 3 or 7 d) and demonstrated increased nitric oxide (NO) secretion, upregulated mRNA for fibronectin, osteopontin, bone sialoprotein and collagen I, with no changes to cell number or viability. The matrix produced by these cells was then decellularised and used to culture C3H10T1/2 cells, a murine MSC cell line. The cells on these matrices were cultured for 7 d with or without LMHFV treatment and exhibited increases in cell attachment, focal adhesions and osteogenic mRNA on the matrices from vibrated cells. The cells also reduced expression of adipogenic mRNA, suggesting the matrix and vibration treatment may promote osteogenesis of recruited MSCs as well as inhibiting adipogenesis. This is in agreement with the *in vivo* work discussed earlier within this review[86,88], providing further evidence for the effects of LMHFV on adipogenesis in MSCs.

Rat BMSCs have also been seeded into scaffolds derived from human bone and subjected to LMHFV (0.3*g*, 40 Hz, ± 50 μm) for 30 min every 12 h, up to 2 d[89]. Cells were found to attach to the scaffolds and produced higher levels of ALP than those cultured in 2D. LMHFV decreased the cell proliferation rate (measured on days 7 and 10), but increased expression of Cbfa1, ALP, col I and OCN. They also studied extracellular signal-regulated kinase 1 and 2 (ERK1/2) involvement and found that ALP activity was reduced when the ERK1/2 pathway was inhibited.

Short bouts of LMHFV (5 min, 5, 30, 60 or 100 Hz) of varying amplitude were shown to promote the release of NO and PGE_2 in MC3T3-E1 cells[90], molecules involved in the *in vivo* response of bone to mechanical loading. The study found no changes in cell shape, alignment or detachment with any of the treatments. When measured 30 min post vibration, there were correlations with increasing maximum acceleration for NO (increasing) and PGE_2 (decreasing) secretion. mRNA for Cyclooxygenase 2 (COX-2), a signalling molecule downstream of PGE_2, was also increased 2 fold by 100 Hz vibration compared to 5 and 30 Hz and 1.5 fold *vs* 60 Hz. It may be important to note that the vibration in this case was applied along the plane of cell attachment, whereas in many studies the LMHFV are applied perpendicular to this. It is not yet know whether this may produce differential effects as there have been no studies directly comparing the two planes of vibration.

After one hour treatment of MLO-Y4 osteocyte-like cells with LMHFV (0.3*g*, 30, 60 or 90 Hz), Lau *et al*[91] found increases in the amount of mRNA for COX-2, particularly at a 90 Hz vibration condition (344% increase). mRNA levels of receptor activator of nuclear factor kappa-B ligand (RANKL), an osteoclastogenic factor, as well as the soluble receptor protein RANKL and PGE_2 secretion, were found to be reduced in the vibrated cells. Conditioned media from the vibrated cells was also found to inhibit osteoclast formation (number of multi-nucleated cells present) and resorption. This suggests that LMHFV may be able to stimulate osteocytes to inhibit resorption in response to the LMHFV. Li *et al*[92] investigated the effects of low intensity pulsed ultrasound (LIPUS) on the interaction between osteocytic and pre-osteoblast like cells. They used 1.5 MHz stimulation on MLO-Y4 murine osteocyte-like cells (3 mm from the LIPUS source) and placed the conditioned media onto MC3T3-E1 pre-osteoblastic cells. Twenty four hours after vibration they saw increased NO and PGE_2 secretion in the MLO-Y4 cells. Conditioned media (collected one hour post vibration) inhibited proliferation in the MC3T3-E1 cells, also causing an increase in the ALP activity of these cells, suggesting osteoblastic differentiation.

Work looking directly at osteoclast precursors found a possible inhibition of osteoclast formation[93]. They used murine osteoclast-like cells RAW264.7 and applied LMHFV (4 Hz, 20 μm displacement) for one hour a day over 3 d and examined the mRNA and protein levels for DC-STAMP (dendritic cell-specific transmembrane protein) and the P2X7 receptor P2X7R, both membrane bound markers of osteoclast fusion. They saw inhibition of mRNA and protein production for DC-STAMP, which suggests the inhibition of osteoclast fusion had occurred. In a model of a BRU using scales taken from goldfish and placed in culture, LMHFV of varying acceleration (0.5, 1, 2, 4, 6*g*) for 5 or 20 min per day showed changes in osteoblast and osteoclast activity after 6 and 24 h[94]. The ALP activity in scales was found to

increase with increasing acceleration, accompanied by a decrease in osteoclast activity (measured by tartrate-resistant acid phosphatase activity). These results were evident at 24 h, with the largest decrease in osteoclast activity for 2*g*. However, although the goldfish scale contains both osteoblasts and osteoclasts, it has a very different macrostructure to that of bones making it difficult to compare with other model systems.

Other studies have compared the effects of LMHFV with high magnitude strain (HMS) on the adipogenic differentiation of MSCs. Sen *et al*[95] used C3H10T1/2 cells and 90 Hz vibration (< 10 microstrain) compared to low frequency HMS (0.17 Hz, 20000 microstrain). They compared pauses within the loading regimen to longer periods of vibration and followed adiponectin expression as a measure of adipogenesis. They found that HMS over 2 or 6 h prevented adipogenesis in the cells, as well as two 20 min bouts separated by at least one hour rest period. The LMHFV was only able to suppress adipogenesis with the two 20 min bouts separated by more than one hour, with a 3 h pause proving better than one or two hours. The inclusion of indomethacin to induce adipogenesis inhibited the effects of the LMHFV but not the HMS treatment. The addition of BMP-2 promoted an osteogenic response with the LMHFV treatment.

A further study was carried out investigating the effects of LMHFV (termed sub sonic vibrations) on 3T3-L1 pre-adipocytes, an embryonic derived cell line[96]. This study used horizontal vibration looking at cell proliferation (3 d, 10, 20, 30 and 40 Hz) and adipogenic maturation (6 d, 10, 20, 30 and 45 Hz). They found that 20 and 30 Hz LMHFV decreased cell proliferation and increased adipogenic gene expression. Increased triglyceride levels within cells were seen for the 10, 20 and 30 Hz vibration conditions. Although the work discussed previously[86-88] showed a reduction in adipogenic gene expression in MSCs, this evidence suggests that LMHFV may increase adipogenesis in cells that are already of the adipose lineage, which would not be a desirable effect *in vivo*. The *in vivo* data on obesity and LMHFV, which demonstrated a lack of reduction in already obese animals[85,86], seems to confirm that LMHFV cause more activity in cells of the adipose lineage, but may be able to mitigate their commitment to this lineage initially.

Interestingly and contradicting the theory that vibration promotes osteogenesis; MSCs from rat bone marrow were found to have lower matrix mineralisation and mRNA for osterix after 6 d period of vibration[97] compared to controls. The cells were subjected to vibration at 0.3*g* and 60 Hz for one hour per day (6 d with one rest day). There were no changes to cell proliferation, ALP activity or mRNA levels for a variety of osteogenic genes (ALP, col-I, OPN, BSP, RUNX-2, OCN). The group hypothesise that, rather than having a direct effect on MSC differentiation, LMHFV may have anabolic effects on bone by causing osteocyte signalling, which in turn affects MSC differentiation. However, many of the *in vivo* studies which have looked at differentiation of MSCs following LMHFV have found increased osteogenic behaviour. This may be due to the differences in cellular environment and vibration transmission between 2D cultures and cells in the 3D *in vivo* environment.

C2C12 murine myoblasts were found to undergo changes following short periods of LMHFV stimulation (5, 8 or 10 Hz, 0.4 mm amplitude, 10 min/d)[98]. After 3 d, expression of collagen Ⅰ and decorin were increased for the 8 and 10 Hz vibration conditions. MyoD and myogenin expression was seen to increase in a time- and dose-dependent manner. There were no changes observed in the cell cycle, but 8 and 10 Hz vibration increase myotube number and length (6 and 9 d of vibration). Vibration also increased the fusion of myocytes in a dose dependant manner. This suggests that the cells are promoting vascularisation, which may aid tissue regeneration *in vivo*.

Much of the *in vitro* work discussed above shows beneficial effects on osteogenic cells, which may lead to the beneficial effects seen in some animal models *in vivo*. The lack of interaction between different cell types however means that it is difficult to know whether the effects seen *in vitro* can help direct therapies *in vivo*. Transmission of LMHFV through muscle, fat and bone may alter the effects on MSCs and differentiated cells, leading to reductions in the efficacy of treatment compared to cell signalling responses and differentiation markers seen in cultured cells.

HUMAN CELLS *IN VITRO*

Reported effects of vibration on human cells are summarised in Table 1. As well as the large number of studies on animal cells and cell lines, there is a growing body of work using human cells and cell lines to investigate the possible mechanisms of action of LMHFV. SAOS-2 cells, a human osteosarcoma cell line, were subjected to 30 Hz vibrations (11 mm displacement) once per day for 4 d[99]. The cells displayed decreased proliferation and increasing mRNA levels for ALP, collagens Ⅰ and Ⅲ, osteonectin and fibronectin, suggesting more osteogenic behaviour. Further work by the group looked at the osteoblastic differentiation of human adipose derived MSCs[100]. They applied LMHFV at 30 Hz to cells for 28 d and found evidence of improved matrix calcification at day 21. They also noted strong increased in the expression of collagen I and osteopontin on day 14 in the vibrated samples when cultured in osteogenic medium, which was not maintained at 21 d. Slightly higher total protein levels were found in vibrated samples compared to non at 21 d, but this effect was lost by 28 d. As many *in vivo* studies do not show any benefits of LMHFV in healthy patients, it is possible that the therapy affects disrupted bone balance, but does not provide any further benefit when normal

Table 1 Summary of effects of low magnitude, high frequency vibration on human cells *in vitro*

Prè *et al*[100]	Adipose derived MSCs	30 Hz, 45 min/d, 28 d	Increase in col-I and osteonectin expression in vibrated group on day 14, lost by day 21. Increased mineralisation in vibrated group at 21 d, lost by day 28
Kim *et al*[102]	MSCs (2 donors)	0.3*g*, 30 Hz for main study. Applied for 10 min/d, 5 d a week for 21 d	Vibration increased ALP activity (days 7, 14 and 21), mRNA (ALP, osteopontin and VEGF) upregulated at day 7. No changes (bone sialoprotein or osteonectin mRNA). In osteogenic media, LMHFV increased the calcium deposition in cultures
Uzer *et al*[103]	Adipose derived MSCs	30 and 100 Hz, 0.15, 1 and 2*g*. 30 min per day for 3-14 d	Cell number increased over 3 d for all conditions. Mineralisation at 14 d increased for 100 Hz and 30 Hz (2*g*). Prevented cytoskeletal remodelling to negate the effects
Zhang *et al*[104]	Peridontal ligament stem cells	0.3*g* (10, 20, 30, 40, 50, 60, 90 and 120, 150 and 180 Hz), 20 min/d to 5 d	LMHFV decreased proliferation, ALP activity highest at 30 Hz. Osteocalcin expression was highest at 40, 50, 60, 90 and 120 Hz. Levels of col-I, RUNX-2 and osterix increased at 40 and 50 Hz
Wolchok *et al*[105]	laryngeal fibroblasts	100 Hz and 3.4*g* (1 s vibration followed by 2 s pause), 6 h followed by an 18 h rest for 21 d	Enhanced secretion of TGFB-1, fibronectin, collagen, increased construct stiffness. Increased gene expression of range of matrix molecules
Cho *et al*[106]	Umbilical cord stem cells	10, 20, 30 and 40 Hz, continuous for 5 d	Increased mRNA and protein levels (MAP2, NF-L and NeuroD1). Time dependant increases in O4 and ERK (12 h at 40 Hz)
Choi *et al*[107]	Adipose derived MSCs	10, 20, 30 and 40 Hz, continuous for 4 d	30 Hz increased astrocyte, oligodendrocyte and neuronal markers, inhibited adipogenesis. Increasing frequency reduced cell proliferation. Time dependant changes in ERK phosphorylation
Edwards and Reilly[101]	hES-MP 002.5 cells	0.3*g* and 15, 30, 45, 60 Hz, one bout 4 min	Increased ALP activity at 60 Hz 48 h post vibration

LMHFV: Low magnitude, high frequency vibration; VEGF: Vascular endothelial growth factor; ALP: Alkaline phosphatase; ERK: Extracellular regulated protein kinases.

bone formation is achieved. The ability to accelerate the initial healing or rebalance of bone formation still has the potential to be a useful therapy.

Our own research with LMHFV and a mesenchymal progenitor cell line, hES-MP 002.5 (Cellartis) has shown promising effects on osteogenic differentiation in 2D[101]. We found evidence that LMHFV at 0.3*g* was able to affect the cell number and osteogenic activity of these cells in 2D culture. Forty-eight hours following a single 45 min bout of vibration, ALP activity was significantly increased for vibration at 60 Hz. This was not seen at lower frequencies tested or 24 h post vibration.

Kim *et al*[102] investigated the use of LMHFV for osteogenic differentiation of hMSCs from two different donors. They applied vibration for 10 min per day (5 d per week to 21 d) at a variety of frequencies and accelerations (10, 20, 30 and 40 Hz and 0.1-0.6*g* in 0.1*g* increments) before choosing 0.3*g* and 30 Hz for the main experiments. Cell proliferation was affected by vibration in a dose dependant manner, with increases in cell number at 30 and 40 Hz conditions on day 2. This increase was not maintained at 5 d however. ALP activity was increased in vibrated samples on days 7, 14 and 21, with mRNA for ALP, osteopontin and VEGF also upregulated at day 7. There were no changes to bone sialoprotein or osteonectin mRNA at this timepoint. When cells were cultured in osteogenic media, LMHFV increased the calcium deposition in cultures, suggesting the vibration has increased osteogenesis.

With LMHFV *in vitro*, there is the likelihood of shear stress occurring in the culture plate due to the movement of media within wells. Uzer *et al*[103] used LMHFV to apply shear stress to human adipose derived stem cells for 30 min a day (3-14 d). They looked at several vibration conditions (0.15, 1 and 2*g*, frequencies of 30 and 100 Hz) and characterised the resulting shear stress (0.04-5 Pa). Over the first 3 d of culture, cell number increased for all conditions, with the greatest increases seen for the conditions with lowest induced fluid shear. After 14 d, mineralisation was increased for all accelerations at 100 Hz and for the 2*g* acceleration at 30 Hz. By disrupting cytoskeletal remodelling, they demonstrated that MSC differentiation was not only regulated by shear stress (as occurred in low stress conditions), but is influenced by cytoskeletal remodelling.

Work by Zhang *et al*[104] on human peridontal ligament cells studied a large range of frequencies (10, 20, 30, 40, 50, 60, 90 and 120, 150 and 180 Hz) at 0.3*g* acceleration. They applied the vibration stimulus for 20 min per day for up to 5 d. The study found decreased proliferation and a frequency dependant increase in osteogenic markers. ALP activity was highest at 30 Hz, while osteocalcin expression was higher at 40, 50, 60, 90 and 120 Hz. Levels of col-I, RUNX-2 and osterix increased at 40 and 50 Hz.

Other research studying horizontal vibration investigated the effects of paused vibration on human laryngeal fibroblasts[105]. This study involved vibration at 100 Hz and 3.4*g* (1 s vibration followed by 2 s pause) for up to 21 d. Vibration was applied for 6 h followed by an 18 h rest and was shown to increase gene expression for matrix and matrix related molecules. These included TIMP1, TIMP3, col1 and col IX, lysyl osidase, TGFB1, syndecan, laminin, connective tissue growth factor and PDGF. In addition, there was enhanced secretion of TGFB-1, fibronectin, collagen and an increase in construct stiffness for low values of strain.

In one study[106], LMHFV (termed sub sonic vibration) was applied at a variety of frequencies (10, 20, 30 and 40 Hz) to human umbilical cord MSCs continuously for

5 d. In this case, the group was interested in neuronal differentiation and found increased in both mRNA and protein levels for several factors including MAP2, NF-L and NeuroD1. They also found time dependant increases in O4 and ERK for 12 h of LMHFV at 40 Hz. A second study[107] assessed the neuronal differentiation of human adipose-derived MSCs, applying LMHFV at the same frequencies over 4 d. RT-PCR was used to investigate both neuronal and adipogenic markers with upregulation at 30 Hz and found that astrocyte, oligodendrocyte, and neuronal markers were significantly increased while inhibiting adipogenesis. Increasing frequency was shown to reduce the cell proliferation. They also noted time dependant changes in ERK phosphorylation, concluding that the LMHFV promoted differentiation to astrocytes and oligodendrocytes through activation of the ERK pathway.

CONCLUSION

The appeal of low magnitude, high frequency vibrations to act as a mechanical stimulus and improve a range of functions within the MSK system has led to an ever growing body of research. The technique is relatively simple to apply, often needs only short periods of stimulation to show some effect and is suitable for those with fragile bones or limited motion. The response varies between different studies, patients and conditions, however, making it problematic to define a predictable set of responses. For healthy subjects, there are often no apparent benefits to the treatment, which suggests that the LMHFV stimulus provides similar mechanical cues to normal daily activities in healthy patients and therefore provide no benefit to bone mass or structure unless there is an underlying condition. There are many studies showing benefits of LMHFV using an acceleration of 0.3*g* or 45 Hz vibration, suggesting these conditions may be particularly effective for the MSK system. Higher frequency vibration, such as 90 Hz, has also show some efficacy at low accelerations (0.2*g*). Evidence is building to suggest that accelerations higher than that due to gravity (> 1.0*g*) may be undesirable, causing some negative effects on bone and mild discomfort in human patients.

In vitro investigation of LMHFV has also produced a wide variety of data, with new research being published at an increasing rate. As with the *in vivo* studies, the range of conditions tested, outcomes measured and goals of the research is very varied, making it difficult to identify common trends. There is increasing evidence that LMHFV has the power to affect the differentiation of stem cells, particularly MSCs. When more of this data is made available, it may be possible to understand how LMHFV might improve the MSK system and aid in the repair of other tissues in the body. Work with primary human cells from multiple donors may provide insights into which patients may be most likely to benefit from LMHFV treatments when conducted alongside *in vivo* investigations. There is also a need for more human trials based directly on small animal studies, using the same outcome measures where possible, to further our understanding of the applicability of these animal models and their relevance to human treatment with LMHFV. Both types of work may help stratification of the therapy to target patients who are likely benefit from the treatment. In general, the use of LMHFV to improve the condition of the MSK system in patients who are not able to maintain high levels of activity or exercise therapy shows promise, but there is still much work to be done on understanding the mechanisms, side effects and benefits to patients.

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