INTRODUCTION:
For elderly, osteoporosis increases the number of fragility fractures. Our previous animal study showed low-magnitude, high-frequency vibration (LMHFV), providing noninvasive and systemic mechanical stimuli, was one of the biophysical modalities which can promote both normal and osteoporotic fracture healing (1). Vibration was also reported to be effective in enhancing systemic blood circulation in normal bone (2). It is well known that a good vascular supply was a prerequisite for the initiation of fracture repair, and angiogenesis is crucial for fracture healing (3,4), especially in early phase of the process. Therefore, we hypothesized LMHFV could increase angiogenesis in both normal and osteoporotic fractures, and hence accelerate fracture healing. Our purposes were to investigate the effects of LMHFV treatment on angiogenesis in normal and osteoporotic fractures and to study the differences in angiogenesis and the possible causes of delayed fracture healing in osteoporotic bone.

METHODS:
9-month old ovariectomy (OVX)-induced osteoporotic rats were randomized into control (OVX-C) or vibration group (OVX-V); age-matched sham-operated rats were assigned into control (Sham-C) or vibration group (Sham-V). 5 days after closed femoral fracture surgery, LMHFV (35Hz, 0.3g) was provided to the treatment groups 20min/day and 5days/week, while sham treatment was given to the control groups. Assessments were evaluated at 3 time points (2, 4, 8 weeks post-treatment), in terms of: Pulsed-wave doppler examination was performed to measure fractured femoral artery blood flow velocity. At the end-points of treatment, animals were anesthetized and the vascular system was perfused with Microfil (Flow Tech, Carver, MA). Then the femurs were harvested to perform microCT scanning using microCT 40 (Scanco Medical, Basserdorf, Switzerland). 3D image of vasculature were reconstructed for quantitative analysis. After microangiography, the decalcified specimens were subjected to histology sectioning and were used for immunohistochemistry to evaluate VEGF (vascular endothelial growth factor) signals. ANOVA and Bonferroni multiple comparison test were used for comparing the above mentioned measurement variables among different groups at different time points. The temporal changes among different groups were analyzed. Significance level was set at p≤0.05.

RESULTS:
(1) Pulsed-wave doppler showed the trend of higher femoral blood flow velocity in vibration groups than control groups. At the 8 weeks time point, significant inferior level of blood flow velocity was found in osteoporotic rats as compared with corresponding normal ones, with p=0.003 (Sham-V > OVX-V) and p=0.005 (Sham-C > OVX-C) respectively. (Fig. 1)

(2) Microfil perfusion & microCT analysis also confirmed increased vessel volume (VV) within callus (OVX-V > OVX-C, p=0.06) and ratio of vessel volume to total tissue volume (VV/TV) in vibration groups than controls at week 4, with a higher increasing percentage in osteoporotic groups (121.2%) compared with normal groups (57.6%). (Fig. 2a, b)

(3) Immunohistochemistry assessment also found higher level of VEGF expression in vibration groups than controls in early phase of fracture healing. (Fig. 3a, b)

DISCUSSION:
Osteoporotic rats had inferior femoral blood flow and vibration groups showed faster velocity than control ones. Reasons might be osteoporosis had decreased capability of vessel formation. LMHFV had the effect of thixotropism which might play a role to decrease the blood viscosity (3). Vibration could also widen small vessels in muscles to reduce the peripheral resistance, heighten muscle activity and muscle metabolic demand by activating muscle spindle reflexes (4,5) and thereby increased the speed of blood flow. LMHFV also promoted angiogenesis within callus in both normal and osteoprotic groups. This might be vibration increased the blood flow shear forces at vascular endothelium due to the inertia of blood, which augmented the functions of VEGF by up-regulating its receptor (6). Percentage of increase in angiogenesis by LMHFV on osteoporotic fracture healing (57.6%) was higher than non-osteoporotic aged (Sham) fracture healing (121.2%). In conclusion, LMHFV could enhance blood flow and angiogenesis at fracture site with different extent in normal and osteoporotic rats, which indicated that promotion of angiogenesis might be the major mechanism of accelerating fracture healing by vibration.

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