INTRODUCTION
Fracture healing is impaired in osteoporotic conditions, accompanied with less-active chondrogenesis [1], prolonged endochondral ossification [2], porous callus structure [3], and decreased mechanical outcomes [4]. Potential enhancements are imminently needed. Providing noninvasive and systemic biophysical stimuli, low-magnitude high-frequency vibration (LMHFV) has been proven osteogenic for osteoporosis [5]. Besides, our previous studies have testified that LMHFV can promote chondrogenesis and endochondral ossification in nonosteoporotic fractures [6]. This study was to test the hypothesis that LMHFV would enhance osteoporotic fracture healing in a rat model.

METHODS
Sixty-one SD rats were ovariectomized at 6 months old, and then underwent closed femoral fracture at 9 months. A reduction in bone mineral density (BMD) was confirmed by peripheral quantitative computed tomography before fracture. After fracture, the animals received vibration treatment (35Hz, 0.3g, 20min/day, 5days/week) or sham treatment since day 5 postoperatively. Follow-up X-ray was taken weekly with healing status graded by two independent clinical surgeons. Callus width (CW) and area (CA) were then measured in lateral radiographs. Besides, six rats from each group were euthanized at 2, 4 and 8 weeks post-treatment for histomorphometric analysis (Safranin-O staining). The total callus area (Cl.Ar) and the cartilage area (Cg.Ar) were quantified separately in the region of interest covering 3 mm around the fracture line. Mechanical property was measured as endpoint assessment using 4-point-bending test. The quantitative data were compared between groups with independent t-test using SPSS software.

RESULTS
Radiographies showed significantly increased CW and CA in the vibration group (VG) than the control (CG) during the first 3 weeks post-treatment (Fig.1). After reaching the peak values, CW and CA decreased from week 4 to week 8, without showing any statistically significant differences between groups. VG showed a higher rate of healing than the control (100% vs. 71.4%) after 8 weeks of treatment.

Histologically, VG presented significantly larger Cl.Ar and Cg.Ar than CG at 2 week post-treatment. At 4 weeks post-treatment, Cg.Ar and Cl.Ar increased in both groups. Significantly larger Cl.Ar and Cg.Ar were observed in VG. At 8 weeks post-treatment, Cg.Ar and Cl.Ar were observed to decrease in both groups. In VG, significantly lower Cl.Ar was observed than that in CG. Meanwhile, there were no statistically significant differences in Cg.Ar between groups.

Mechanical testing showed that the ultimate load of the femur in VG was 23.1% higher (p<0.05) than that of CG (Fig. 2). The stiffness showed 9.9% higher values (p<0.05) in VG than in CG. The energy to failure was 69.9% higher (p<0.05) in VG as compared with CG.

DISCUSSION
This study demonstrated that LMHFV promoted callus formation and led to higher rate of healing and better mechanical outcomes in osteoporotic fractures. Compared with our previous studies on nonosteoporotic fracture healing [6], the healing process was significantly prolonged in the present study. Both early reaction of periosteal bone formation, as reflected in radiographic followup, and the capacity of chondrogenesis, as shown in histomorphometry, were impaired in osteoporotic fracture healing compared with the nonosteoporotic one.

The enhancement of fracture healing by LMHFV was probably due to a stimulated callus formation from both intramembranous and endochondral origin. During the callus formation stages, an increased callus formation was reflected by radiographic quantification. The radiopaque callus was confirmed to be subperiosteal woven bone in histological observations, and hence was generated from intramembranous ossification of the periosteum. On the other hand, histomorphometry confirmed an enhanced chondrogenesis during callus formation in VG, as reflected by significantly larger Cl.Ar and Cg.Ar at 2 week post-treatment, which was followed by a promoted endochondral ossification. These observations illustrated not only the potency of LMHFV to stimulate callus formation, but also the complexity of the underlying mechanism that played a role. The detailed mechanism concerning how the functional cells sense and respond to the mechanical stimuli remains to be investigated.

In conclusion, all these evidences support that LMHFV promotes both intramembranous and endochondral bone formation, enhances osteoporotic fracture healing, and hence lead to improved mechanical outcomes. LMHFV possesses high potential as a noninvasive enhancement for osteoporotic fracture healing clinically.

ACKNOWLEDGEMENT
RGC Earmarked Grant CUHK 4502/06M; AIOD Research Grant 131006 KSWH

REFERENCES

Fig.1. Weekly monitoring of callus width (CW) and area (CA) in lateral radiographs. VG showed increased callus formation during the first three weeks, followed by a faster decrease of CW and CA from week 4 to week 8.

Fig.2. Mechanical properties of the fractured femora after 8 weeks of LMHFV or sham treatment. VG showed better mechanical outcomes than CG.