A novel single pulsed electromagnetic field stimulates the osteogenesis and angiogenesis — in vitro and in vivo study
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ABSTRACT INTRODUCTION:
Pulsed electromagnetic field (PEMF) has been successfully applied for accelerating fracture repair as early as 1979. Since the treatment required 8-12 hrs of application daily, the inconvenience leads us to develop a newly designed single pulsed electromagnetic field (SPEMF). This device is capable of enhancing osteogenesis with human bone marrow derived mesenchymal stem cells (hBMSCs) with 3 min/day treatment. In this study, thymidine incorporation was used to examine cell proliferation. Alkaline phosphatase activity assay and mineralization assay were used to evaluate osteogenic differentiation of hBMSCs and previous bone graft mice model to confirm in vitro study. Our results showed that the cell proliferation was enhanced in SPEMF-treated groups at day 2 and 4 of treatment. The ALP activity of hBMSCs was significantly increased in SPEMF-treated groups after 2-5 days of treatment. The mineralization was also increased after 15-25 days of SPEMF stimulation. Furthermore, a short-term treatment to hBMSCs with SPEMF for 7 days also achieved the same effect of SPEMF stimulation at 15th-25th days. In vivo results showed both of the osteogenesis and neovascularization significantly improved in SPEMF groups. In conclusion, the newly developed SPEMF can accelerate the osteogenesis and dead bone revascularization. The potential benefit is that fracture healing is being promoted by early and short application of SPEMF after fracture or osteonecrosis.

METHODS:
The single pulsed electromagnetic field (SPEMF) is in composition of a single pulsed repeated in adjustable times and magnetic fields. Pulsed period is 5 ms in sine wave per stimulation. The magnetic field is produced by every single pulse and the magnitude of magnetic field is adjustable in range from 0.6 Tesla up to 1 Tesla (1 Tesla is equal to 10^4 Gauss). Every pulse needs 5 s to storage energy for next pulse and 30 times per day for 5 days to examine whether SPEMF would lead to cytotoxicity of hBMSCs or not. Bone marrow derived mesenchymal stem cells were harvested from the iliac crest of adult donors. After 5 days treatment, the media and the cell lysate were analyzed for LDH activity using a cytotoxicity detection kit (Roche®) to evaluate whether SPEMF would lead to cytotoxicity of hBMSCs or not. The effect of SPEMF on proliferate rate of hBMSCs was detected at day 2 and day 4. On the 2nd and 4th day, 1 mcI/well of [H] thymidine was added to each well and incubated for 4 hr. Alkaline phosphatase (ALP) activity was thought as a marker for osteogenic differentiation of hBMSCs. In addition, Alizarin Red S stain was performed to observe the effect of SPEMF on mineralization of hBMSCs. Mineralization was determined by Alizarin Red S stain. In vivo study, soft X-ray observation, histological and immunostaining analysis of bone tissue were performed.

RESULTS SECTION:
The data shows that SPEMF would not lead to cytotoxicity in all three cases of four conditions after five days stimulation. After the stimulation of SPEMF, the proliferative rate increased significantly up to 31% at day 2 (** p < 0.01), and 29% at day 4 (* p < 0.05) of treatment. Under an osteogenic environment of hBMSCs, the ALP activity was significantly increased after SPEMF stimulation (Fig 2). Mineralization of hBMSCs was significantly increased in SPEMF 1-25 treatment specimen at 15th day, and the effect lasted until 20th day and 25th day. A short-term stimulation, SPEMF 1-7 achieved similar effect and was compatible with SPEMF 1-25 (Fig 3). In vivo, our both SPEMF groups showed better treatment effect than control. On week 2, we could see bridging callus formation over posterior aspect ofibia and only little radiolucent gap over anterior tibia potion. On week 4, they eventually united all and we could not see any difference between SPEMF 1-7 and SPEMF 1-25 groups (Fig 4). In H&E stain, after 4 weeks, callus bridged the fracture area and around the graft bone on all groups and the total callus area amount had no difference in all groups. However, Table 1 showed that the percentages of bone matrix in SPEMF 1-7 and 1-25 groups had significant difference compared to control. After the treatment with SPEMF, the formation and growth of new blood vessels displayed brown color by IHC stain. Table 2 showed that there were the percentages of lacunae with cells encapsulated in SPEMF 1-7 and 1-25 groups had significant difference compared to control.

DISCUSSION:
In this study, first, we have demonstrated that our SPEMF did not lead to cells cytotoxicity on hBMSCs. Second step, we also have proved that our novel electromagnetic field was not only increased proliferation of hBMSCs, osteogenic differentiation of hBMSCs, but also accomplished the desire goal in less than 3 min of treatment per day. Final step, we have demonstrated the same results in vivo to confirm our in vitro study. In conclusion, our result showed that SPEMF would increase proliferation of hBMSCs in a proliferative environment, increased differentiation of hBMSCs in an osteogenic environment, and accelerated mineralization in a mimetic physiologic environment. In vivo, we use animal model to prove all in vitro findings. Therefore, we can conclude that SPEMF could be a useful device in enhancing fracture healing and early osteonecrosis treatment.

REFERENCES: