Low Intensity Pulsed Ultrasound Treatment on Spinal Fusion Augmented with Tissue Engineered Biomaterial Composite

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Objective:
Low intensity pulsed ultrasound (LIPUS) has been found to enhance fracture healing (1), distraction osteogenesis (2,3) and also autograft implanted for spinal fusion in rabbit model (4). Little has been done on the effects of LIPUS on tissue engineered bone and others bone substitutes. This study investigated the effect of LIPUS on the tissue engineered biomaterial composite in a rabbit spinal fusion model.

Methods:
Bone marrow was aspirated from proximal femur of New Zealand white rabbits (15 week old). The mesenchymal stem cells (MSC) in passage 2-3 were then cultured and expanded in number with DMEM/osteogenic supplements/basic fibroblast growth factor for 1 week. The composites of MSC ($5X10^6$ cells) impregnated beta-tricalcium phosphate blocks (TCP) were implanted on decorticated L5 and L6 transverse processes in a rabbit posterior spinal fusion model. LIPUS was applied for 20min daily on the back of rabbit for 7 weeks (LIPUS group, n=7) starting from day 5 postoperatively. The MSC group (n=7) comprised of rabbits without LIPUS treatment and the control group with only cell free TCP block implant (Control group, n=7). Two fluorochromes, xylenol orange and calcein were injected subcutaneously into rabbit back at week 4 and 6 to label the newly formed bone. The spinal segments were harvested at week 7 and assessed by manual palpation, microCT assessment, peripheral quantitative computed tomography (pQCT), histology, histomorphometric of contour area of fluorochrome labeled bone inside marocpores of TCP implant).

Results:
Manual palpation showed solid fusion in 86% (6/7) of LIPUS group comparing with 14% (1/7) in MSC group and 0% (0/7) in control group (Table 1). 3D microCT images showed radiological inter-transverse process bony fusion beneath the TCP block only in the LIPUS group (Fig. 1 and Table 1). The volume of transverse processes in LIPUS group was 38.3% and 22.1% greater than the control and MSC group respectively (Table 1). Histologically, bony fusion was observed in inter-transverse process interval in LIPUS group along the interface of TCP block but not in MSC and control group (Fig. 2). In the histomorphometry analysis, the MAR of LIPUS group was 36.1% and 31.0% greater than the control and MSC group respectively (Table 1). Numerous newly formed osseous tissues were integrated into macropores of TCP block (Fig.2). The osteointegration area of LIPUS was 1.8 fold greater than control and 3.8 fold than MSC group (Table 1).

Discussion:
This was the first study on the effectiveness of LIPUS in enhancing solid fusion in posterior spinal fusion implanted with tissue engineered stem cell-tricalcium phosphate bioceramics. LIPUS augmented bone formation rate and also the fusion mass. The LIPUS exerted micromechanical stress to stimulate osteogenesis by enhancing proliferation and differentiation of osteoblast (5). It also promoted bone matrix formation and mineralization (6) thus facilitating bone formation. Moreover, LIPUS was found to enhance bony integration by respectively to label the newly formed bone. It might help to enhance rigidity of the fusion mass (8).

Conclusion:
Non-invasive low intensity pulsed ultrasound enhanced tissue engineered stem cell-bioceramics implanted posterior spinal fusion. It has the important advantage of reducing the morbidity associated with autograft harvesting and potential clinical applications in extensive posterior spinal surgery requiring bony fusion.

Table 1

	LIPUS	MSC	Control
Manual Palpation	86%	14%	0%
Radiological fusion	86%	0%	0%
Volume of transverse processes (mm^3)	581.8 ± 36.3*	420.7 ±50.7*	476.5 ± 15.9*
MAR (μm/week)	28.1 ± 3.1$^{\#,*}$	21.4 ± 3.3 $^{\#}$	20.6 ± 3.2*
Osteointegration area (mm^2)	28.04 ± 7.10*	5.85 ± 1.94*	10.11 ± 2.42*

*p<0.01, $^{\#}$ p<0.05 by ANONA and Bonferroni post-host test

Fig. 1. 3D microCT reconstructed image of LIPUS, MSC and control group

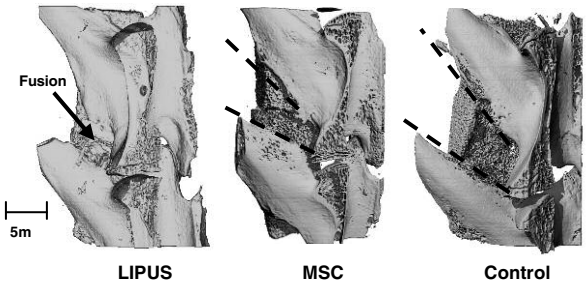
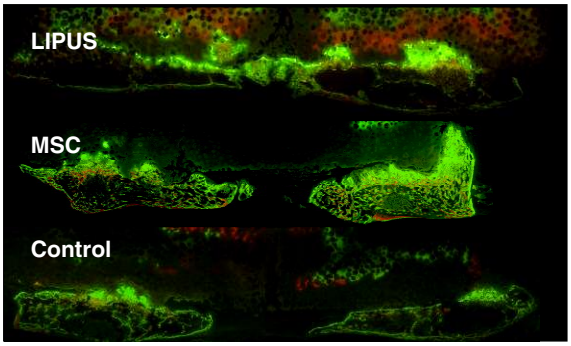


Fig. 2. Epi-fluorescent histology of LIPUS, MSC and control group



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