Low Intensity Pulsed Ultrasound (LIPUS) Accelerates Systemic Recruitment of Mesenchymal Stem Cells (MSCs) for Fracture Healing

1Chin, W C; 2Leung, K S; 3Li, G; 3Qin, L; 4Cheung, W H
+The Chinese University of Hong Kong, Hong Kong, China, 3Institute of Biomedical and Health Engineering, Shenzhen, China
email: louis@ort.cuhk.edu.hk

ABSTRACT INTRODUCTION:
Fracture healing is a biological regenerative process that follows a well-orchestrated sequence. It starts with an inflammation phase followed by the formation of a callus filled by granulation tissue. The callus is then invaded by mesenchymal stem cells (MSCs) and blood vessels. Therefore, the recruitment of multi-potent MSCs to differentiate into sufficient osteogenic cells in early stage of fracture repair is crucial for a successful fracture healing. With recent research showing the efficacy of low intensity pulsed ultrasound (LIPUS) on increasing cellular activities as well as enhancing blood flow\(^1\), we hypothesized that LIPUS could enhance the systemic recruitment of MSCs for fracture healing. The objectives of this study were to investigate the effect of LIPUS on intracardially administered MSCs homing to fracture site and to evaluate its efficacy to accelerate fracture healing.

METHODS:
In this study, sixty 3-month-old rats were used to create closed fracture at femur shaft, according to our established protocol\(^2\). The fractured rats were divided into 3 groups: (i) intracardiac injection of GFP-labelled MSCs (GFP-MSCs) plus LIPUS group (MSC-LIPUS), (ii) intracardiac injection of GFP-labelled MSCs group (MSC), and (iii) no-treatment control group (CTL). For the MSC-LIPUS and MSC groups, 4 million GFP-MSCs (in 0.5 mL by volume) was delivered intracardially on day 3 post-fracture. The rats in MSC-LIPUS group were further given daily LIPUS treatment following the cell injection since day 3 post-fracture. Euthanasia time points were at 1, 2, 3 and 4 weeks after fracture. To monitor the MSCs homing to fracture sites, end-point GFP fluorescence on the rat femur ex vivo with imaging system (IVIS 200, Xenogen, USA) and immunohistochemistry. Weekly radiology, micro-computed tomography and histomorphometry were assessed on monitoring the fracture healing progress.

RESULTS SECTION:
GFP fluorescence measurement (at harvest) showed both the MSC-LIPUS and MSC groups were with higher values than that of the CTL group at all the time points, despite no significant difference.

Table 1: The end-point GFP fluorescence measurement (in counts x10^6)

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSC-LIPUS</td>
<td>2.6±0.73</td>
<td>2.6±0.12</td>
<td>2.8±0.33</td>
<td>2.8±1.0</td>
</tr>
<tr>
<td>MSC</td>
<td>2.8±0.61</td>
<td>2.6±0.64</td>
<td>3.0±0.16</td>
<td>2.5±0.48</td>
</tr>
<tr>
<td>CTL</td>
<td>1.8±0.39</td>
<td>1.7±0.41</td>
<td>1.9±0.57</td>
<td>2.0±0.84</td>
</tr>
</tbody>
</table>

Immunohistochemistry result showed the amount of injected GFP-MSCs present at the fracture site, an indication on the possible homing effect (Figure 1). The MSC-LIPUS group was shown to have the highest signal at all the time points among the groups. And within the MSC-LIPUS, the GFP-MSCs signal was the highest at Week 2.

DISCUSSION:
This is the first study to show LIPUS could enhance MSCs homing. Moreover, the enhanced fracture healing process under LIPUS was as well demonstrated. Through the GFP fluorescence measurement and immunohistochemistry assessment, the positive relationship between the application of LIPUS and the accelerated homing effect of the injected MSCs to the fracture site was illustrated. From the radiology, microCT and histomorphometry assessments, the MSC-LIPUS group was shown with the fastest woven bone bridging rate; the much enhanced endochondral ossification during the fracture healing process. The use of LIPUS was shown with an enhancing effect on fracture healing. As previous study showed LIPUS stimulates the synthesis of chemoattractant for MSCs homing\(^7\), further study on the CXCR4/SDF-1 pathway could elucidate the possible mechanism by which the MSCs are mobilized and recruited under influence of LIPUS.

SIGNIFICANCE:
This study shows the use of LIPUS on enhancing fracture healing, as well as providing an insight on the mechanism of MSCs homing under LIPUS; a possible alternative approach for non-union which the cellular activities are low and local MSCs have been depleted.

ACKNOWLEDGMENT:
This study was supported by OTC grant (Ref. No. 2009-WHLG)

REFERENCES:

---

**Figure 1:** HRP-staining of the immunohistochemistry (200X)

**Figure 2:** Radiology (callus width)

**Figure 3:** Histomorphometry (16X)

**Figure 4:** micro-CT (Qualitative)

**Figure 5:** micro-CT (Quantitative)