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● *Original Contribution*

MICROBUBBLE INJECTION ENHANCES INHIBITION OF LOW-INTENSITY PULSED ULTRASOUND ON DEBRIS-INDUCED PERIPROSTHETIC OSTEOLYSIS IN RABBIT MODEL

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Abstract—We determined whether the addition of microbubbles enhances the effect of low-intensity pulsed ultrasound (LIPUS) on bone–implant integration in an early-stage osteolysis model. The bone canals were injected with titanium particles before implantation to establish the periprosthetic osteolysis model. Before ultrasonic therapy, the microbubble-enhanced LIPUS group ($G_{Ti\text{-}Us\text{-}Mb}$) received an intra-articular injection of microbubbles. Biomechanical testing revealed that $G_{Ti\text{-}Us\text{-}Mb}$ had significantly greater fixation strength than the LIPUS group ($G_{Ti\text{-}Us}$). Distal periprosthetic bone mineral density was also higher in $G_{Ti\text{-}Us}$ than in the Ti group (G_{Ti}), but no significant increase was detected after administration of microbubbles. Histomorphometric analyses revealed that bone formation around the implant in $G_{Ti\text{-}Us}$ was enhanced by the addition of microbubbles in $G_{Ti\text{-}Us\text{-}Mb}$. Taken together, our data indicate that microbubble injection enhances the inhibitory effect of LIPUS on debris-induced osteolysis and further strengthens the mechanical fixation of implants in an early-stage osteolysis model *in vivo*. (E-mail: jointsahzu@aliyun.com)

Key Words: **Implant osseo-integration, Microbubble, Low-intensity pulsed ultrasound, Osteolysis.**

INTRODUCTION

Aseptic loosening is a major complication and a leading cause for failure of arthroplasty (Harris 2001). Clinical strategies to improve implant fixation include design modifications of modular components, such as highly cross-linked polyethylene and ceramics, delivery of local or systemic pharmaceuticals, such as bisphosphonates (Morris and Einhorn 2005) and advanced surgical techniques. However, a favorable approach for orthopedic physicians is lacking because of the progressive nature of periprosthetic osteolysis once it occurs; therefore, revision surgery remains indispensable for end-stage patients. Despite being the most effective method, by far, for the treatment of aseptic loosening, surgical revision is associated with the potentially devastating complication of periprosthetic joint infection. Thus, there is great interest in developing alternative, non-invasive approaches to inhibit periprosthetic osteolysis at the beginning of its course.

Experiments on ultrasound for the stimulation of fracture healing had been conducted for a long time before its approval by the U.S. Food and Drug Administration in 1994. Since then, research exploring the prospects for its applications has not stopped. Ultrasound also has been reported to have positive effects in the osseo-integration of metal implants as well as in osteoradionecrosis (Liu et al. 2012; Wu et al. 2013). Recently, Zhao et al. (2012), for the first time, reported the effect of low-intensity pulsed ultrasound (LIPUS) (1.5 MHz, 200 mW/cm^2) on periprosthetic osteolysis *in vivo*; they revealed that LIPUS could inhibit osteolysis induced by polyethylene, resulting in a thinner fibrous membrane at the interface as well as better fixation strength (Zhao et al. 2012). This provided new insight into inhibition of the biological process of particle-induced osteolysis and the formation of an interface membrane and, thus, indicated the ability of ultrasound to inhibit the early stages of osteolysis through a non-invasive approach.

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Microbubbles were first introduced as an ultrasound contrast agent for vascular imaging. Within the last decade, many studies have addressed the increasing applications of microbubble-mediated ultrasound for therapeutic uses (Ibsen et al. 2013; Qiu et al. 2012). These beneficial applications generally depend on the ability of microbubbles to promote ultrasonic cavitation and sonoporation. Recently, ultrasonic ablation of synovial pannus through microbubble-enhanced cavitation was performed successfully (Qiu et al. 2012). Ultrasonic cavitation and related bio-effects are considered to cause microvascular endothelial injury and subsequent thrombosis, which induces further apoptosis and necrosis of cells in the target tissue. The interface membrane is a major pathologic lesion found in revision arthroplasty of aseptic loosening, and typically, the tissue is highly vascularized and fibrous and rich in wear particles, fibroblasts, macrophages and multinucleated giant cells (Yang et al. 2011). The biological characteristics of an interface membrane are similar to those of synovium; thus, it is also regarded as a synovium-like membrane with an invasive nature (Goldring et al. 1983; Jones et al. 1999; Pap et al. 2001). We hypothesized that microbubble-enhanced ultrasound could exert similar ablative effects on an interface membrane. We supposed that, combined with the beneficial effects of LIPUS on periprosthetic osteolysis, microbubble-enhanced LIPUS would improve the mechanical fixation of implants in a periprosthetic osteolysis model.

In the present study, we sought to optimize the inhibitory effects on the process of debris-induced osteolysis and to improve further the mechanical fixation of implants in an established periprosthetic osteolysis model by introducing microbubbles.

METHODS

Experimental design

A model of periprosthetic osteolysis was created by local injection of endotoxin-free titanium particles (concentration: 1.2×10^8/mL) into the femoral medullary canal of a rabbit before a titanium plug was press-fitted, similar to previous studies (Yang et al. 2007; Zhao et al. 2012). Titanium particles were re-injected at the first, third and fifth weeks postoperatively. In total, 48 hindlegs of 24 male New Zealand rabbits (mean weight: 3.7 kg, range: 3.5–4.2 kg) were randomly divided into four groups. The first group did not receive titanium particles (G_{Con}, n = 12); the three other groups did. The second group received titanium particles as a positive control (G_{Ti}, n = 12). The third group received a 0.3-mL saline injection and LIPUS treatment ($G_{Ti\text{-}Us}$, n = 12), and the last group ($G_{Ti\text{-}Us\text{-}Mb}$, n = 12) received a 0.3-mL intra-articular injection of sulfur hexafluoride-filled microbubble solution (volume concentration: 7–8 μL/mL, mean diameter: 2.5 μm, 90% were <6.0 μm, 99% were <11.0 μm; Sonovue, Bracco, Geneva, Switzerland) before ultrasound treatment. All procedures were approved by the Animal Care and Use Committee of the Second Affiliated Hospital of Zhejiang University.

Titanium plug and particle preparation

Ti_6Al_4 V plugs (4 mm in diameter, 20 mm in length) with an acid-etched surface were provided by the Experimental Research Center of Mechanics, Zhejiang University, and were sterilized after ultrasonic cleaning. Titanium particles with a mean diameter of 3.0 μm (93% <20 μm) were purchased from Alfa Aesar (Stock No. 00681, Ward Hill, MA, USA). Particles were sterilized with 95% ethanol in a magnetic stirrer for 24 h at room temperature. A limulus assay (chromogenic TAL endpoint assay kit, Xiamen Houshiji, Fujian, China) was used to assay endotoxin levels and to ensure they were below 0.25 EU/mL. After being washed in phosphate-buffered saline (PBS) and dried, particles were resuspended in PBS at 1.2×10^8/mL with 100 U/mL penicillin and 100 U/mL streptomycin and stored at 4°C (Bi et al. 2001; Zhu et al. 2010).

Animal model of titanium particle-induced osteolysis

Pentobarbital sodium (3%, 1 mL/kg) was used for anesthesia during surgery. The knee joint was exposed through a medial parapatellar approach, and a bone canal (3.8 mm in diameter and approximately 20 mm in length) was created using a 3.8-mm-diameter, low-speed surgical drill at the intercondylar notch. Then, 600 μL of Ti particle suspension was injected into the Ti(+) canal, and an equal volume of carrier solution was injected into the Ti(−) canal. The plug was press-fitted immediately into the slightly undersized canal after administration of Ti particles or carrier solution. Each rabbit received an implant in each femur. The incision was closed with interrupted absorbable sutures (4-O Coated Vicryl *Plus, Ethicon, Johnson & Johnson Medical, Shanghai, China). No restriction was imposed on walking (Jinno et al. 2002). In the first, third and fifth weeks after surgery, the titanium particle suspension (600 μL) was re-injected into the knee joint cavity of all animals, with the exception of those in the G_{Con} group. Penicillin was administered at 800,000 U daily for 3 d postoperatively. All animals were euthanized by pentobarbital sodium overdose 6 wk after implantation. Femurs containing implants were isolated and stored in gauze moistened with PBS at −20°C and underwent biomechanical testing within 24 h. One case of unilateral fracture and one of infection were replaced.

Fluorescence bone labeling

Sequential administration of polychromatic fluorescent dyes was performed as reported previously (Van