Antibacterial Properties of Ultrafine-grained Biomaterials

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INTRODUCTION: Implant-related infection is one of the most serious complications following instrumentation. Many studies have reported the technology of implants with antibacterial properties. These technologies often use coating and surface modification to inhibit biofilm formation, which is the critical pathogenesis of postoperative infection. However, widespread adoption has been limited due to concerns about biocompatibility, potential cytotoxicity, and the emergence of antibiotic-resistant bacteria.[1] We focus on an ultrafine-grained material for the prevention of implant-related infection, which has a heterogeneous surface consisting of various nano-scale particles, resulting in greater mechanical strength while maintaining high biocompatibility due to no change in its composition.[2] Our preliminary studies showed a significant reduction in bacterial adhesion on the flat material surface *in vitro*, suggesting the potential antibacterial effect *in vivo*. This study aims to evaluate the antibacterial properties of ultrafine-grained stainless-steel material for the prevention of implant-related infection. We developed a novel mouse model of implant-related infection that allows the evaluation of two types of materials in the same individual and quantitatively assessed biofilm formation on the surface of ultrafine-grained stainless-steel wire (grain size of 500 nm) compared to conventional wire (grain size of 1000-1500 nm) over time.

METHODS:
in vitro assay: Five stainless-steel wires (8 mm length and 0.5 mm diameter; ultrafine-grained wire or conventional wire) were placed in a 24-well plate containing a 1000µL culture of 1.0×10^8 CFU/mL bioluminescent *Staphylococcus aureus* (Xen36). Plates were incubated in a shaking incubator at 37 °C, and samples were collected at 1, 3, and 7 days post-culture.
in vivo assay: All animal experiments followed protocols approved by the Institutional Animal Care and Use Committee (IACUC). We modified the reported spinal-implant-related infection mouse model [3]. Two types of L-shaped wires (3mm x 5mm) were placed on both sides of the L4 spinous process. 10µL of 1.0×10^5 CFU/mL Xen36 culture was injected into subfascial layer after the implantation (Fig. 1). Samples were collected at 1, 3, 7, and 10 days after surgery.
Biofilm evaluation: The biofilm formation on the wire surface was assessed by crystal violet staining. Quantitative analysis of the bacterial numbers in the biofilm was performed by colony forming units (CFU) counting. Quantitative analysis of the biofilm maturation was performed by the gene expression profiles of early biofilm forming-related genes (icaA and clfA).

RESULTS: In vitro, the ultrafine-grained wires exhibited a marked decrease in biofilm, stained by crystal violet, on the wire surface after overnight culture compared to the conventional wires (Fig. 2), and CFU counting on day 1 showed a significant decrease in the ultrafine-grained wire. The expression of both icaA and clfA was lower in the ultrafine-grained wires on day 3, with icaA showing a statistically significant difference. In vivo analysis, CFU counting demonstrated that there were significant less bacteria on the ultrafine-grained material after 3 days of surgery (Fig. 3).

DISCUSSION: Our findings indicate that ultrafine-grained stainless-steel wire may possess antibacterial properties, resisting the initial adhesion of bacteria and subsequently inhibiting biofilm formation on its surface. In clinical settings, where prophylactic antibiotics are administered perioperatively, even if the effect is limited to the early phase, it might help antibiotics in eradicating contaminated bacteria before forming mature biofilm, which leads to decrease implant related infections. Without altering their chemical compositions, ultrafine-grained materials offer both antimicrobial properties and biocompatibility, potentially addressing unmet clinical needs for orthopaedic implants.

SIGNIFICANCE/CLINICAL RELEVANCE: Given the negative impact of surgical site infections on patients and health outcomes, this biomaterial could address a significant clinical need by reducing the risk of postoperative implant-related infections. Hence, ultrafine-grained metal materials, which do not require any surface modification and coating, may be a novel technology to replace traditional materials for surgical implants.

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3. Kelley BV, Zoller SD, Greig D, et al. In vivo Mouse Model of Spinal Implant Infection. J Vis Exp. 2020 Jun 23;(160)

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Figure.1 Representative images of a novel in vivo mouse model of implant related infection.

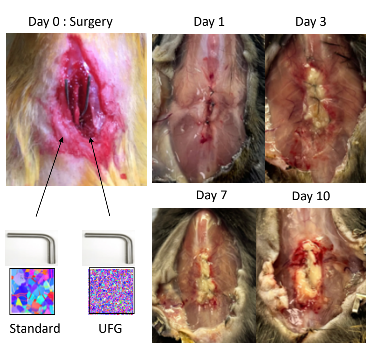

Figure. 2 Infected wires are stained with crystal violet one week after culture in vitro. (Left image) Conventional stainless-steel wire (n = 4); (Right image). Ultra-fine-grained stainless steel (UFG) wire (n = 4).

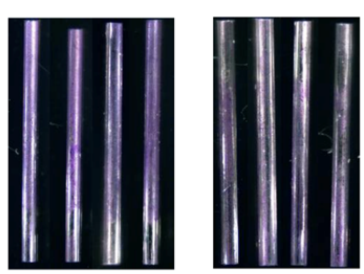

Figure.3 In vivo comparison of the colony forming units (CFU) between the conventional wire sand the ultra-fine-grained stainless steel (UFG) wire on day 3. Data presented as mean values ± SEM (n=9/group, p=0.05)