Antibacterial Function of Novel Peptide Incorporated Titanium Alloys and Feasibility of This Surface Treatment for Orthopaedic Implantations

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Introduction: Titanium alloy has been widely used in orthopaedic implantations due to its excellent corrosion resistance and biocompatibility[1]. Nevertheless, the success of these implants is hindered by bacterial infections[2]. Bacterial adhesion on implant surface is an important step in the pathogenesis of implant-related infections. Therefore, preventing initial bacterial adhesion on implant surfaces is important to avoid biofilm formation and subsequent bacterial infections[3].

Several approaches including surface loaded with antibiotics[4], titanium oxide[5], silver and copper ions[6] have been reported. Release of the loaded ions is involved in the killing of bacteria. Regardless of efficient killing, shortcomings such as durability of the release mechanism and compatibility of the released ions with surrounding tissues are still unresolved.

In this study, we aim to achieve the reduction of bacterial adhesion by incorporating novel peptides on the Ti surfaces. To demonstrate its feasibility for bone surgery, the cytocompatibility of the treated surface treatment has been also investigated by using osteoblasts.

Methods: 5mm diameter medical grade Ti-6Al-4V alloy discs were first oxidized to Ti-OH[7] and then converted to Ti-OSi-NH_2[8]. Afterwards, our novel peptides 1 or 2[#] were covalently incorporated onto the Ti surfaces.

Antibacterial function of the treated surfaces was evaluated by counting the number of adhered live bacteria on the substrate. The samples were incubated with $6x10^4$ *Staphylococcus aureus* cells for 30 min. After washing away the non-attached bacteria cells, the samples were stained *(Molecular probes L7012)* and the number of live bacteria on the fluorescent micrograph was counted.

The cytocompatibility of our surface treated Ti alloys was investigated by adhesion test and MTT cell viability assay. Primary green fluorescent protein mice osteoblast (GFP-OB) was used for the adhesion test. 4,000 cells were applied onto each disc and the cell morphology was examined under fluorescent microscopy after 10h incubation. For the MTT assay, 30,000 MC3T3-E1 pre-osteoblasts cells were applied onto each sample and the standard MTT procedures were conducted. The absorbance values were measured by a microplate reader.

Results:

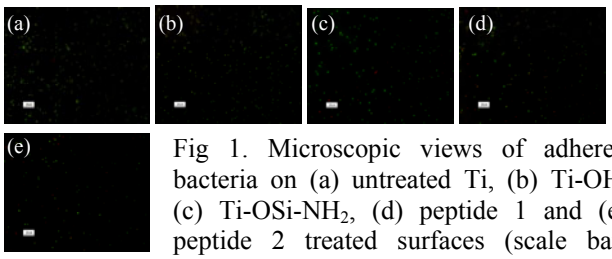

Fig 1. Microscopic views of adhered bacteria on (a) untreated Ti, (b) Ti-OH, (c) Ti-OSi-NH_2, (d) peptide 1 and (e) peptide 2 treated surfaces (scale bar: 20μm). Green and red spots represent live and dead bacteria respectively. The number of live bacteria reduced by 23% and 74% respectively in peptide 1 and 2 treated surfaces compared with untreated control.

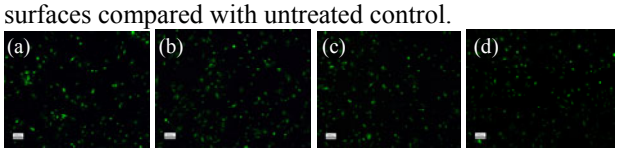

Fig 2. GFP-OB adhered on (a) untreated Ti, (b) Ti-OH, (c) Ti-OSi-NH_2 and (d) peptide 2 treated surfaces (scale bar: 200μm). The micrographs show that osteoblast can adhere and grow well on all tested surfaces.

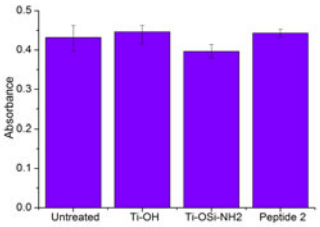

Fig 3. MTT results for untreated Ti, Ti-OH, Ti-OSi-NH_2 and peptide 2 treated surfaces. The absorbance value for all treated surfaces was similar to the untreated Ti surfaces, indicating a lack of cytotoxicity in all treated surfaces.

Discussion: The number of live bacteria on peptide 1 treated surfaces were reduced by 23% in the bacterial adhesion test. By modifying the peptide sequence, we demonstrated a 74% reduction in peptide 2 treated surfaces. We believe that the alteration of the peptide sequence has contributed to the enhancement of the antibacterial properties of the peptides, which is also reported in other studies[9, 10].

Conclusion: While the primary objective of this study is to improve the antibacterial activity of the Ti implant, the biocompatibility of this novel surface treatment is also concerned. Both our MTT assay and osteoblast adhesion test prove the lack of cytotoxicity of our treatment. Our novel surface treatment is potentially useful in orthopaedic implantations to resist bacterial infections. For the future development, the relationship between the peptide sequence and antibacterial activity will be investigated and long-term effect on mammalian cells will be evaluated by *in-vivo* experiments.

References: [1]Bruni S. Acta Biomaterialia. 2005;1:223-234. [2]Darouiche RO. New Engl J Med. 2004;350:1422-1429. [3]Campoccia D. J Microbiol Meth. 1997;30:141-152. [4]Radin S. Biomaterials. 2007;28:1721-1729. [5]Chung CJ. Surf Coat Tech. 2007;202:1302-1307. [6]Wan YZ. Vacuum. 2007;81:1114-1118. [7]Chu CL. J Biomed Mater Res Part A. 2005;75A:595-602. [8]Martin HJ. Langmuir. 2007;23:6645-6651. [9]Bagheri M. Antimicrob Agents Chemother. 2009;53:1132-1141. [10]Haynie SL. Antimicrob Agents Chemother. 1995;39:301-307.

[#] *Patent pending*