

Enhanced bone formation on newly fabricated Cu-bearing stainless steel

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Statement of Purpose: Stainless steel is commonly used in orthopaedic implantations because of its high strength and toughness as well as ease of processing [1]. However, its osteoconductivity does not satisfy the demands in bone-implant integration, thereby resulting to aseptic implant loosening and eventually implant failure [2,3,4]. In addition, higher rate of implant-related osteomyelitis was occurred in stainless steel [5]. Therefore, a new type of stainless steel incorporating with nano-sized copper-rich participations in the matrix has been fabricated. In past, this newly fabricated steel had demonstrated its superior antimicrobial effect in-vitro and in-vivo [6-8]. The present study aims at investigating the adhesion and proliferation as well apoptosis of osteoblastic cells on Cu-bearing stainless steel, the osteogenic differentiation ability of osteoblasts against to the release profile of copper ions, and the bone formation ability as well as inflammatory response under in-vivo condition.

Methods: The samples of 317L-Cu stainless steel (Cr 19, Ni 13, Mo 3.5, Cu 4.5 and Fe in balance in wt%) for in-vitro tests were prepared from rods in 4.4 and 13.9 mm diameter to disks with 1 mm in thickness and followed by mechanical polishing until mirror surface. For in-vivo testing, samples were machined into cylindrical shape of 2mm in diameter and 6mm in length and followed by electrochemical polishing. Conventional 317L stainless steel (SS) served as control. To determine the amount of Cu²⁺ ions released, samples were submerged to physiological saline and incubated under 37°C bio-incubator from D0 until 1 month. The extracts were then examined by inductively coupled plasma atomic emission spectrometer. Material characterizations included electrochemical corrosion analysis, scanning electron microscopy and energy dispersive spectrometry. The cell viability, adhesion, proliferation and apoptosis as well as the expression of osteogenic differentiation markers (i.e. alkaline phosphatase (ALP), type I collagen (Col1- α 1), runt-related transcription factor 2 (Runx2) and osteopontin (Opn)) on sample surface were investigated by culturing SaOS-2 human osteoblasts and MC3T3-E1 mouse pre-osteoblasts for various periods of time, respectively. In in-vivo, the samples were implanted into the rat models until post-op 15 days. Post-op analyses included bone quality evaluation and new bone formation monitored by micro-computed tomography at D3, 7 and 15, separately. Also, histological and immunohistochemical analyses were applied to examine the new bone formation and inflammatory response of tissues adjacent to the implants. Push-out biomechanical test was conducted in order to assess the mechanical strength of bone-implant interface. All data were analyzed by Student's t-test using ANOVA and statistical significance would only considered when level of p was < 0.05.

Results and discussion: It reported that more actin filaments were found on Cu-SS sample than that of SS control in the morphology examination after 2-day culture. The cell proliferation and the expression of ALP, Col1 α 1, Opn and Runx2 of osteoblasts in Cu-SS group were significantly higher as compared with the SS. In addition, the LDH assay

suggested that the cytotoxicity was even lower than that of SS. Furthermore, higher volume of new bone formation adjacent to the implant and bone-to-implant contact ratio were reported in the Cu-SS sample under in-vivo condition. The BMD of newly formed bone on Cu-SS sample was higher. Additionally, the expression of TNF- α on Cu-SS sample was significantly less than that of SS sample at Day 3 and 7, indicating that the release of small amount of copper ions could help suppress inflammatory response. The push-out force of Cu-SS group was much higher than that of SS group. This result suggested that the osseointegration at the bone-implant interface in Cu-SS group was superior to that of conventional SS. Lastly, the corrosion testing result indicated that the release of Cu²⁺ ions was triggered by pitting corrosion at the Cu rich phases of the new stainless steel matrix. The amount was approximately 1.4 ppb per day, while its corrosion resistance generally maintained. In summary, in addition to the antimicrobial effect, our current study has demonstrated that small amount of copper ions can not only help promote morphogenesis of osteoblasts and up-regulate the major osteogenic differentiation markers, but also enhances the osseointegration and suppresses the TNF- α expression in-vivo.

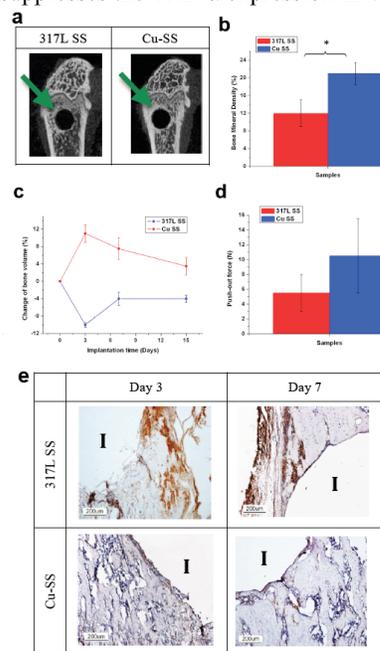


Fig. 1 (a) u-CT images of lateral epicondyl implanted 317L SS and Cu-SS after 15 days of operation. (b) BMD of the bone formed around the implants at D15. (c) Percentage change of the bone volume from D0-15. (d) Average maximum push-out forces of 317L SS and Cu-SS at post-op D15. (e) Immunohistochemical staining of TNF- α of bone tissue around the implants at D3 and D7. Brown color represents positive staining and 'I' represents the implant location.

References: [1] P Elter et al. *Acta Biomater.*, 2009 (5): 1468–73. [2] S Nayak et al. *Biomater.*, 2013 (34): 2855–64. [3] P Tengvall et al. *Biomater.*, 2004 (25): 2133–38. [4] LZ Zhao et al. *Biomater* 2013 (34) 19–29. [5] LG Harris et al. *Injury, Int. J. Care Injured* 37, S3 (2006). [6] L Ren et al. *Mat & Design.* 2011(32): 2374–79. [7] L Ren et al. *Mat Sci and Eng C.* 2012 (32): 1204–09. [8] D Zhang et al. *Col and Surf B: Biointer* 2013 (105): 51–57.

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