

Abstract

Photofunctionalization of titanium enhances bone-implant integration in rat osteoporosis model

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【Abstract】

I . Introduction

Osteoporosis is one of risk factors for implant treatment because it causes bone fragility. Photofunctionalization of titanium has been shown to increase the bone-implant contact rate and bone-implant integration strength. The present study aimed to investigate the effect of ovariectomy on the establishment of osseointegration and the efficacy of photofunctionalization treatment in a rat osteoporosis model.

II . Materials and methods

1. Implant sample analysis

Commercially pure titanium discs were acid-etched with both hydrofluoric acid and sulfuric acid. The control group (NA) was stored in a dark condition for 1 month after the dual-acid treatment. For the photofunctionalization group (PA), NA was irradiated with short-wavelength ultraviolet light for 15 minutes before the measurement. In order to evaluate the wettability, the contact angles of NA and PA were measured.

2. Animal experiments

Cylindrical titanium implants were prepared by the same way mentioned above. For animal experiments, 14-week-old female Sprague Dawley (SD) rats with ovariectomy (OVX rats) and 14-week-old female SD rats without ovariectomy (Con rats) were used. NA and PA implants were placed 9 mm from the distal epiphysis of the left and right femurs in OVX rats, respectively (OVX-NA group, OVX-PA group). Only NA implants were placed in Con rats (Con-NA group). This experiment was carried out with the approval of the Animal Experiment Committee of Health Sciences University of Hokkaido (Approval number 19-053).

3. Biomechanical push-in test

Bone-implant integration strength was evaluated at weeks 2 and 4 post-surgery by biomechanical push-in test. Implant surface after biomechanical push-in test was analyzed using Scanning Electron Microscope (SEM) and Energy Dispersive X-ray spectroscopy (EDX).

4. Micro CT analysis

The bone density (BV/TV, %) and bone-implant contact rate (BIC, %) were determined from micro CT data of the femur including the implant removed at week 4 post-surgery.

5. Histological observation

Undecalcified polishing specimens of the femur including the implant removed at week 4 of healing were stained with Villaneba Goldner, and observed with an optical microscope.

6. Cell culture experiments

Bone marrow-derived mesenchymal stem cells (BMSCs) were isolated and cultured from the femurs of both 14-week-old OVX rats and Con rats. OVX rat-derived cells were seeded on NA and PA titanium discs, and Con rat-derived cells were seeded on NA titanium discs. The cells on titanium were counted using a hemacytometer at days 3 and 14 after seeding, and the ALP positive rate of the cells on titanium was measured at day 12 after seeding.

III. Results and Discussion

1. Ovariectomized rat for osteoporosis model

CT images of Con rats femurs showed thick cortical bone and layered, resinous and dense trabecular structures, whereas CT images of OVX rats femurs showed thinning of the cortical bone and reduced trabecular bone. The graph of body weight change showed that the body weight of OVX rats significantly increased

after week 10 of age, 2 weeks after ovariectomy, compared with that of Con rats. These results confirmed that estrogen deficiency due to ovariectomy induced the pathological conditions similar to osteoporosis.

2. Photofunctionalization of titanium

The contact angle of PA was significantly lower compared with that of NA. Therefore, the photofunctionalization treatment restored the hydrophilic properties of the titanium surface.

3. Osseointegration

In the biomechanical push-in test at weeks 2 and 4 post-surgery, the maximum push-in load for the OVX-NA group was significantly lower compared with that for the Con-NA group. The maximum push-in load for the OVX-PA group was significantly higher compared with that for the OVX-NA group. For the SEM observation and EDX analysis of the implant surface at weeks 2 and 4 post-surgery, the Con-NA and OVX-PA groups showed more bone-like tissue residues, which were similar to hydroxyapatite in composition, compared with the OVX-NA group. For the Micro-CT imaging at week 4 post-surgery, BV/TV and BIC for the OVX-NA group were significantly lower compared with those for the Con-NA group. BV/TV and BIC for the OVX-PA group were significantly higher compared with those for the OVX-NA group. For the histological images at week 4 post-surgery, the OVX-NA group more soft tissue interventions at bone-titanium interface. These results suggested that increased bone formation around photofunctionalized implant might result in the enhancement of bone-implant integration strength in ovariectomized rats.

4. Cell culture experiments

Both the number and ALP positive rate of osteogenic cells for the OVX-NA group were significantly lower compared with those for the Con-NA group. Both the number and ALP positive rate of osteogenic cells for the OVX-PA group were

significantly higher compared with those for the OVX-NA group. Ovariectomy reduced the proliferation and osteogenic differentiation of BMSCs, however photofunctionalization of the titanium implant enhanced the proliferation and differentiation of BMSCs.

IV. Conclusion

This study concluded that enhanced proliferation and differentiation of BMSCs on the photofunctionalized titanium surface could increase the new bone formation and the bone-implant integration strength even in ovariectomized rats.