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Enhanced cell adhesion and proliferation on dual plasma modified titanium surfaces

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Introduction: Ti-6Al-4V is widely used in present orthopedic applications, owing to a combination of good mechanical properties and excellent corrosion resistance. However, long-term success of Ti-6Al-4V implants and the completeness of their osteointegration still need to be addressed [1]. Since the biofunctionality of the implant is strongly affected by its surface characteristics, to promote osteointegration, considerable efforts have focused on modifying the surface of the implants. Alternatively, surface modification using plasma immersion ion implantation (PIII) has been developed in order to incorporate new biofunctional groups onto titanium alloy surfaces [2]. The present study aims at investigating the impact of carbon-nitrogen (Car-Nit) dual plasma surface treatment on bioactivity of Ti-6Al-4V alloy surfaces.

Methods: Ti-6Al-4V discs measuring 5 mm in diameter and 1.5 mm in thickness were prepared and polished to mirror finish. Carbon, nitrogen and carbon-nitrogen dual PIII treatments were applied at an implantation energy of 47 kV, 40 kV and 40 kV, with a radio frequency of 10Hz, 200 Hz and 200 Hz, and pulse width at 500 µs for 2 hours, 30µs for 2 hours and 30µs for 2 hours, respectively. Assessments of surface bioactivity using MC3T3-E1 osteoblasts were conducted. In the cell adhesion assay, 10000 cells were cultured on various sample surfaces for 4 hours. The seeded samples were stained with the aid of LIVE/ DEAD Staining Kit and observed by fluorescence microscopy. The total number of adhered cells was estimated according to the image-sample surface area ratio. Cell proliferation was measured by MTT assay on days 2, 4 and 7 of cell culturing. Quantification of cell number was conducted by measuring the absorbance value of MTT solution at wavelength 570nm using a spectrophotometer.

Results: The samples with dual PIII treatment have a lower water contact angle and more cell attachment as compared to their corresponding single PIII treated samples and untreated sample ($p<0.05$) (Fig. 1-2). The cell viability assay indicates that dual PIII treatment evidently improves osteoblast proliferation (Fig. 3).

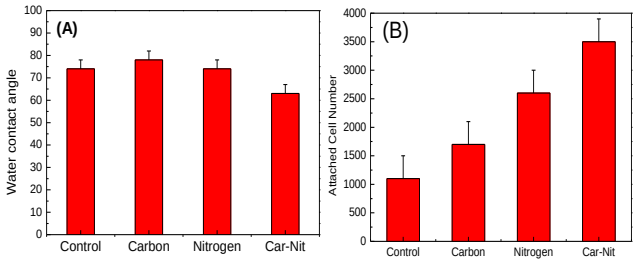

Fig. 1 (A) The water contact angle and (B) Cell adhesion assay results of samples with various treatments, untreated sample serving as control

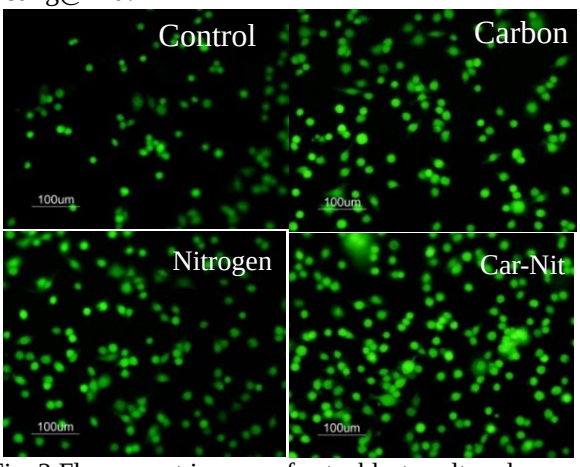

Fig. 2 Fluorescent images of osteoblasts cultured on various samples for 4 hours

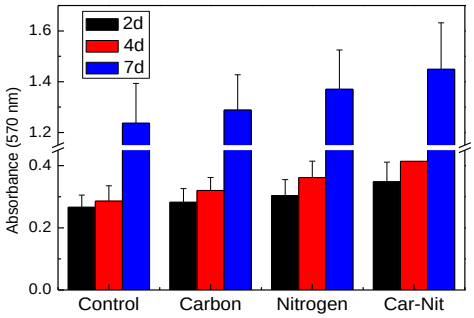

Fig. 3 Cell proliferation results of various samples

Discussion and Conclusion: Based on the results of cell adhesion and proliferation, carbon-nitrogen dual plasma surface treatment can stimulate osteoblast activity at the early stage of cell-material interaction and show better bioactivity as compared with only carbon or nitrogen plasma surface treatment. The bioactivity enhancements may be attributed to the coexistence of carbon and nitrogen functional groups formed at high implantation energy and shown high surface hydrophilicity.

In conclusion, carbon-nitrogen dual plasma immersion ion implantation treatments enhance surface hydrophilicity and promote osteoblast adhesion and proliferation on Ti-6Al-4V alloy surface. High implantation energy can result in a better biological response.

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