ENHANCED CORROSION RESISTANCE AND BIOCOMPATIBILITY BY PLASMA IMPLANTATION OF NICKEL-TITANIUM ALLOYS: AN IN-VITRO AND SURFACE CHEMICAL STUDY

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Introduction: Stainless Steels, titanium, and titanium alloys are the common standard orthopaedic metals. Recently, nickel-titanium (NiTi) shape memory alloys have been proposed as alternatives as implanting materials due to their two unique properties, shape memory effect (SME) and super-elasticity (SE) that may not be found in stainless steels and titanium alloys. A number of recent studies suggest that this material is compatible with living tissues; however, adverse effects have also been reported. For instance, a study found that the osteogenesis process and osteoconectin synthesis activity in NiTi alloys were unfavorable as compared to stainless steels and titanium alloys. Another study reported that the cell death rate was severe on NiTi alloys. This problem is suspected to be due to the poor surface corrosion resistance that may lead to an increase in cytotoxicity. The toxic materials released from the substrate resulted in cell death possibly rather than cell apoptosis. Prior NiTi can be used clinically, the corrosion and wear resistance must be enhanced, since fretting at the interface of coupleings of orthopedic implants is always present. We have therefore employed plasma surface treatment to enhance the corrosion resistance and wear resistance of the material. Our previous results show that these surface properties can be significantly improved using acetylene, nitrogen, or oxygen plasma immersion ion implantation (PIII). The objective of this study is to analyze the surface chemistry of those surface treated materials, untreated NiTi alloys, medical grade 316L stainless steels and commercial medical grade pure titanium and then compare their cytocompatibilities using enhanced green fluorescent protein mice osteoblast cells.

Materials and Methods: Circular NiTi bars with 50.8% Ni were cut into discs of 5 mm in diameter and 1 mm in thickness. Surface polishing was then carried. Nitrogen, oxygen and acetylene were implanted to the surface of NiTi discs. Stainless steel rods and commercial pure Ti rods were prepared in the same way and 5 mm diameter and 1 mm thick. The samples were ground, polished and cleaned under the same conditions before analysis and cell culturing. The surface chemical compositions were determined using the survey scanning mode by X-ray photoelectron spectroscopy (XPS). A monochromatic aluminium source was employed and the acquisition area was 0.8 mm in diameter. Five areas of each sample were randomly selected for analysis. For the cytocompatibility testing, osteoblasts isolated from calvarial bones of 2-day-old mice that ubiquitously express an enhanced green fluorescent protein (EGFP) were used to culture in a Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, antibiotics (100U/ml of penicillin and 100µg/ml of streptomycin), and 2mM L-glutamine at 37°C in an atmosphere of 5% CO2 and 95% air. The specimens (1mm in thickness and 5mm in diameter) were fixed on the bottom of a 24-well tissue culture plate using 1% (w/v) agarose. A cell suspension consisting of 5,000 cells was seeded onto the surface of the untreated NiTi samples, O2 implanted NiTi samples, N2 implanted NiTi samples, C2H2 implanted samples, stainless steel samples, and pure titanium samples as well as wells without any metal discs serving as a control for normal culturing conditions. Cells were grown in 1 ml of medium and changed every three days. Cell attachment was examined after the second day of culture, and cell proliferation examined after 4, 6 and 8 days of culture. There were at each time point four identical samples for statistical comparison. In our study, cells were allowed to reach confluence during the examination period. To determine the cell number, the attached cells were released by digestion with trypsin-EDTA (Invitrogen) and counted using a haemacytometer. Cell viability was assessed by staining with 0.2% Trypan blue (Sigma). The number of cells was expressed as mean value ± standard deviation (SD).

Discussion: Stainless steel, commercial pure titanium and titanium alloy are the most common artificial metals for surgical implantation due to good corrosion resistance, deformability and good compatibility with living tissues. However, someone reported that stainless steels had poor corrosion resistance under physiological conditions that resulted into nickel (Ni) and chromium (Cr) release into the biological medium. Ni is highly toxic to living tissues and reported to be a carcinogenic element as well. Furthermore, Cr may cause impairment of osteoblast proliferation and differentiation in addition to cytokine release. These findings may explain the insignificant growth of osteoblasts on our stainless steel samples in this study, since Cr and Ni were also detected in the surface layer of the samples. Therefore, stainless steel may not be the optimum material for permanent implantation. Alternatively, the use of nickel titanium shape memory alloy in orthopedic implants has been controversial due to its high nickel concentration in comparison to other medical grade metals. Our research groups have proposed the use of surface plasma treatment to enhance the corrosion resistance as well as nickel ion leaching. The surface modified NiTi alloys display better anti corrosion and anti wear-corrosion properties due to new composite layer forming at the surface after plasma ion implantation treatment. Those composite layers are well tolerated by osteoblast cells rather than the medical grade stainless steel and pure titanium. Therefore, the surface treated NiTi alloys are very promising as for orthopaedic permanent implantation.

Results: According to the results of surface chemistry analysis, the major compounds found in the untreated NiTi samples surfaces were TiO, TiO2, and NiO. These compounds were also found in the oxygen-treated samples, but the amount of TiO2 present in the latter was higher than that in the former. For the nitrogen-treated surfaces, TiN, TiO2 and tiny amount of Ni metal were detected, whereas for the acetylene-treated surfaces, titanium carbides (TiCn) and a small amount of Ni metal were found. On the stainless steel surfaces, Fe was the major element with significant amounts of Cr, Mo, and Ni. Some carbides and NiO were also present. For the commercial pure Ti samples, only TiO and TiO2 were found. All plasma-implanted and untreated samples are well tolerated by the EGFP-expressing osteoblasts. The mean and the S.D. values are summarized in the figure below. After culturing for 2 days, cells started to attach to and proliferate on all the samples, except for stainless steel samples. The proliferation on NiTi, nitrogen and oxygen implanted samples were highest among them. After 4 and 6 days, cell proliferation on the untreated NiTi alloy samples was higher than that on the nitrogen, oxygen and acetylene PIII samples as well as other samples such as pure titanium and stainless steel. However, the nitrogen implanted samples exhibited the highest degree of cell proliferation among the samples after 8 days. It should be noted that the rate of cell proliferation on the NiTi samples appeared to level off after 6 days but that on other samples investigated continued to increase gradually. The stainless steel samples exhibited the least amount of cell proliferation over 8 days of cell culturing. The proliferation on the acetylene and oxygen implanted samples was comparable to that on the titanium samples. An insignificant amount of dead cells emerged after 8 days of culturing perhaps due to cell apoptosis.

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