Long Term Cytocompatibility and \textit{In vivo} Investigation of Nitrogen Plasma Implanted Shape Memory Alloy

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\textbf{Introduction}: Nickel ion release in nickel-titanium (NiTi) shape memory alloy impedes its clinical applications in particular to orthopaedic implants in which fretting is always expected at the implant junction. High level of nickel is toxic to the surrounding biological tissues. Therefore, efforts have been made to deal with this concern by applying various surface treatments. Someone has even developed nickel-free shape memory alloys. Previously, we successfully demonstrated the enhancement of biological and surface mechanical properties of NiTi alloys by using plasma immersion ion implantation (PIII) technology. However, the long-term biological effects of these plasma treatments are unknown. This paper therefore characterizes the effect of nickel release upon long-term simulated body fluid (SBF) immersion test to cytocompatibility and \textit{in-vivo} behavior of PIII treated and untreated samples.

\textbf{Methods}: NiTi discs with 50.8\% Ni were treated by nitrogen PIII at 40kV with 100Hz. Long-term biological tests including SBF immersion, alkaline phosphatase (ALP) activity measured by reverse transcription – polymerase chain reaction (RT-PCR) and \textit{in-vivo} animal study were performed up to 12 months.

\textbf{Results and Discussion}: With the use of inductively coupled plasma mass spectrometry analysis, the nickel amount of nitrogen treated samples was found less than the untreated after 2 months SBF immersion. For the results of 12 months, the release of untreated was stabilized and no different as compared with nitrogen treated sample. In long-term enhanced green fluorescent protein mouse osteoblast cell culturing, ALP activity of nitrogen sample exhibited no difference in untreated and medical grade titanium alloy at all time points. Although no significant difference was found in such \textit{in-vitro} tests, the \textit{in vivo} bone formation was found to be better on the nitrogen treated surfaces at every time points.

\textbf{Fig. 1} Intra-operative picture of animal implanting nitrogen plasma treated sample into femur. White arrow indicates the entry point.
Fig. 2 Rate of nickel ion release under SBF solution for about two months. It indicated that the rate of nickel ion release of untreated nickel titanium alloy was higher than that of nitrogen plasma treated nickel titanium.

Fig. 3 Accumulative concentration of released nickel ion release under SBF solution for about two months. It also observed that the total amount of nickel ions of untreated nickel titanium alloy was significantly higher than that of nitrogen plasma treated nickel titanium.

Fig. 4 Alkaline phosphatase expression of enhanced green fluorescent protein mouse osteoblasts cultured with the extracts from untreated and nitrogen plasma treated NiTi at 3 and 12 months (a: control, b: untreated NiTi, c: nitrogen treated post-culture day 3 with 3 months extract; d: control, e: untreated NiTi, f: nitrogen treated post-culture day 3 with 12 months extract; g: control, h: untreated NiTi, i: nitrogen treated post-culture day 7 with 3 months extract; j: control, k: untreated NiTi, l: nitrogen treated post-culture day 7 with 12 months extract)

Fig. 5 Histological analysis of untreated (Left) and nitrogen plasma treated (Right) nickel titanium alloys after 52 weeks of animal implantation (20x magnification). Red color represents to bony tissue. It is obviously suggested that more bony tissue can be aggregated on nitrogen plasma treated sample under long term in vivo condition.

Conclusion: This long-term biological study suggested that nitrogen plasma treated nickel titanium alloy has superior bony on-growth under in vivo condition. The in vivo bioactivity of nitrogen treated sample is comparable to the medical grade titanium alloy.

Acknowledgement: This study is financially supported by Hong Kong Government ITF (GHP #019/05).