J Mater Sci: Mater Med (2007) 18:2263–2268
DOI 10.1007/s10856-007-3118-2

Dissolution effect and cytotoxicity of diamond-like carbon coatings on orthodontic archwires

Shinya Kobayashi · Yasuharu Ohgoe · Kazuhide Ozeki · Kenji Hirakuri · Hideki Aoki

Received: 18 October 2005 / Accepted: 24 July 2006 / Published online: 12 June 2007

Abstract Nickel–titanium (NiTi) has been used for implants in orthodontics due to the unique properties such as shape memory effect and superelasticity. However, NiTi alloys are eroded in the oral cavity because they are immersed by saliva with enzymolysis. Their reactions lead corrosion and nickel release into the body. The higher concentrations of Ni release may generate harmful reactions. Ni release causes allergenic, toxic and carcinogenic reactions.

It is well known that diamond-like carbon (DLC) films have excellent properties, such as extreme hardness, low friction coefficients, high wear resistance. In addition, DLC film has many other superior properties as a protective coating for biomedical applications such as biocompatibility and chemical inertness. Therefore, DLC film has received enormous attention as a biocompatible coating.

In this study, DLC film coated NiTi orthodontic archwires to protect Ni release into the oral cavity. Each wire was immersed in physiological saline at the temperature 37 °C for 6 months. The release concentration of Ni ions was detected using microwave induced plasma mass spectrometry (MIP-MS) with the resolution of ppb level. The toxic effect of Ni release was studied the cell growth using squamous carcinoma cells. These cells were seeded in 24 well culture plates and materials were immersed in each well directly. The concentration of Ni ions in the solutions had been reduced one-sixth by DLC films when compared with non-coated wire. This study indicated that DLC films have the protective effect of the diffusion and the non-cytotoxicity in corrosive environment.

S. Kobayashi · Y. Ohgoe · K. Hirakuri
Department of Electronic and Computer Engineering, Tokyo Denki University, Hatoyama, Saitama 350-0394, Japan

K. Ozeki (✉)
Department of Mechanical Engineering, Ibaraki University, 4-12-1, Nakanarusawa, Hitachi, Ibaraki 316-8511, Japan
e-mail: ozeki@mx.ibaraki.ac.jp

H. Aoki
International Apatite Co., Ltd, 20 Kanda-Ogawamachi, 3-Chome, Chiyoda-ku, Tokyo 101-0052, Japan

Introduction

Nickel–titanium (NiTi) has unique properties, including shape memory effect and superelasticity, which make it an attractive candidate for biomedical applications. NiTi has been used for implants in orthopedics and orthodontics for several decades and is responsible for major improvements in these fields [1]. However, NiTi erodes in the oral cavity under extreme pH and temperature variations, and this can lead to corrosion and nickel dissolution. A primary concern about NiTi is the high nickel (Ni) content of the alloy, and the possible influence of this high Ni content on biocompatibility. However, other concerns exist about implanted material releasing Ni that may generate harmful reactions. For example, Ni may generate allergenic, toxic and carcinogenic reactions following skin contact or implantation of the material [2].

Some investigations have been carried out primarily to inhibit the release of Ni ions from material with biocompatible coating. Ozeki et al. reported that titanium coatings created on NiTi by sputtering techniques protect Ni from release [3].

Diamond-like carbon (DLC) films have excellent properties, such as extreme hardness, low friction coefficients, and high wear-resistance [4], and are increasingly gaining

importance in many forms of industrial application, including wear-resistant coatings for hard-disk drives and optical components, as well as in semiconductor devices [5–7]. DLC film has many other superior properties as a protective coating for biomedical applications, such as biocompatibility and chemical inertness. Therefore, DLC film has received enormous attention as a biocompatible coating [8].

The majority of the few published reports are on the biomedical applications of DLC film. Grill et al. investigated the biocompatibility of DLC film in hip and knee joints [9]. Ohgoe et al. reported on the biocompatibility of DLC film in artificial heart valves and the diaphragm [10]. However, no application of DLC film to dental materials has been reported. We investigated DLC film-coated NiTi orthodontic archwires and the effectiveness of DLC film in preventing Ni release from NiTi archwires.

Materials and methods

DLC films were deposited on NiTi orthodontic archwires (Ni:Ti = 50.50:49.50) (Model TMSJ-09, TOMY International Inc., Japan; 0.46×0.64 mm^2) using arc discharge ion plating process (Model: DASH 860, Nanotec Co., Japan). High benzene gas 99.9999% was used as a reactive gas. The gas pressure in all deposition was 2.3×10^{-5} Pa. The substrate bias in all deposition was 2.0 kV. The samples were ultrasonically cleaned for 15 min in acetone as a pretreatment. The thickness of all samples at 1 μm was maintained by using the roughness measurement (Model: SJ-400, Mitutoyo Co., Japan).

Mechanical brushing test

The effect of mechanical force on the adhesion between the DLC film and NiTi archwires was assessed using a mechanical brushing machine with a toothbrush (Model: K654, Tokyo Giken Ltd., Japan)(Fig. 1). The wire coated with DLC film was polished constantly for 350 min at a load of 35 g, which simulated the daily brushing of teeth over a 6-month period. After the polishing, the surface of the wires was observed by a Raman spectrophotometer (Raman: model NRS-2100, JASCO Co., Japan) using an argon laser (514.52 nm) at 1 mW.

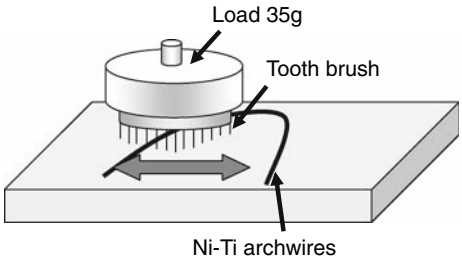

Fig. 1 Schematic drawing of the mechanical brushing

Immersion test

The purpose of this study was to evaluate the DLC films, which serve to increase the stability of the surface layer of the archwire by protecting the bulk material from corrosion. In this test, DLC-coated wires and non-coated wires were prepared. All samples were immersed in physiological saline at a constant temperature.

Firstly, three DLC-coated wires and three non-coated wires were constantly immersed in physiological saline for 14 days at 70 °C. The protective effect of the DLC film was determined by measuring Ni release. The release of Ni ions was detected by microwave introduced plasma mass spectrometry (MIP-MS: model P-6000, Hitachi, Ltd., Japan) with ppb-level resolution. The concentration of Ni was measured by detecting Ni ions as a function of immersion time.

The same six sample wires were then immersed in physiological saline at a constant temperature of 37 °C. After 6 months, the concentration of Ni ions was measured and the corrosion resistance of each sample was assessed by observing the surface appearance under SEM. The molecular composition of the integuments, formed by immersion of the surface of wires, was determined by micro Fourier transform infrared spectroscopy (micro FTIR: model FT/IR-620, Jasco, Japan). Spectra were acquired on an FTIR spectrometer equipped with an infrared microscope attachment (model IRT-30, Jasco Co., Japan) operating under the following conditions: 3,200–2,500 cm^{-1} range, and 4 cm^{-1} resolution.

Cytotoxicity test

Squamous carcinoma cells derived from human oral cancer tissue (Sa3, RIKEN, Japan) were cultured in 175 cm^2 tissue culture flasks (Falcon 353028, Becton, Dickinson and Co., New Jersey USA) in Eagle's minimum essential medium (E-MEM) supplemented with 10% fetal bovine serum (FBS, GIBCO 1017, Invitrogen Corp., Carlsbad, CA, USA) at 37 °C in 95% CO_2 humidified atmosphere. After being washed in phosphate-buffered saline solution (pH = 7.4, PBS), adherent cells were detached from culture flasks by addition of 2 mL 2.5%trypsin/2% ethylenediaminetetraacetic acid for 5 min at 37 °C. These Sa3 carcinoma cells were then seeded in 24-well culture plates (Falcon 353047, Becton, Dickinson and Co., New Jersey USA) at a density of 2×10^4 cells/well.

Samples (10 mm long) of DLC-coated and non-coated wires were immersed in each well containing the Sa3 carcionma cells. Sample wires were sterilized by autoclave at 121 °C for 20 min before immersion. A 100% E-MEM solution that did not contain any samples was prepared as a control. Cells were counted with a Neubauer hemocytometer (Neubauer hemocytometer: Model A116, Sun Lead Glass Co. Ltd., Tokyo, Japan).

After 96 h of cell culture experiments, the cells remaining on the sample wires were counted and their morphology was observed using SEM. For SEM, the samples were fixed in 2.5% glutaraldehyde for 24 h and then transferred through a 50–100% ethanol gradient into 1% osmic acid fixative and vacuum-dried prior to gold sputtering.

Results

Mechanical brushing test

The Raman spectra of DLC-coated wires before and after polishing are shown in Fig. 2. Each of the wires had a spectrum that was typical of the DLC Raman spectrum at any position, exhibiting a shouldered peak (D-band at 1,350 cm^{-1}) and a broad peak (G-band at 1,570 cm^{-1}). This indicated that DLC coatings exist without exfoliating against mechanical brushing.

Immersion test

Figure 3a shows a graphical representation of the release of Ni ions from NiTi archwires in physiological saline as a function of a 14-day immersion time. The release of Ni ions was inhibited to undetectable levels, less than 50 ppb, by DLC films. However, the release of Ni ions from non-coated wires was more than 1.5 ppm on the five days, and gradually increased with subsequent immersion time. Figure 3b shows the comparison of Ni dissolution for each sample after immersion for 6 months. The release of Ni ions from non-coated wire was 0.93 ppm. In contrast, Ni release from DLC-coated wire was 0.15 ppm. The concentration of Ni ions released in the solution was reduced by one-sixth using DLC coating compared with non-coated wire.

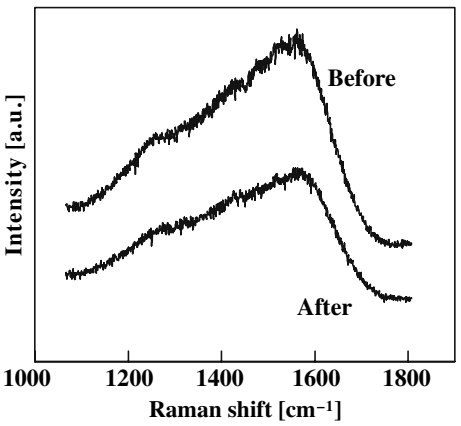

Fig. 2 Raman spectra of polished and non-polished wires

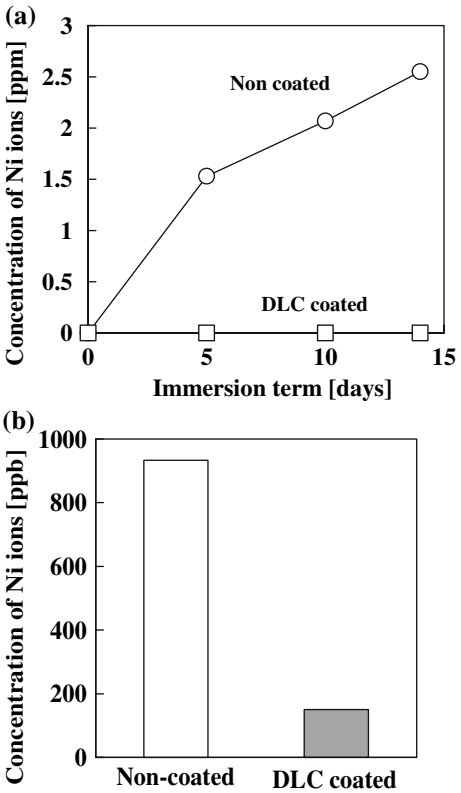

Fig. 3 (**a**) The release of Ni ions from NiTi archwires in physiological saline as a function of a 14-day immersion time (**b**) Concentration of Ni ions released from DLC-coated wire and non-coated wire immersed in physiological saline for 6 months

Figure 4 shows SEM images of the surfaces of the archwires before and after immersion in physiological saline for 6 months. The surfaces of DLC-coated wires showed no difference before and after immersion. However, the surface of non-coated wires before immersion was significantly smoother and more homogeneous than after immersion.

Micro-FTIR spectra obtained from the surface of the DLC wires before and after immersion in physiological saline for 6 months clearly showed no difference (Fig. 5). The fine structure of the C–H stretch absorption spectrum yields quantitative information on the fraction of sp^3 and sp^2 hybridization in the carbon network. The predicted C–H stretch frequencies are shown Table 1 [10].

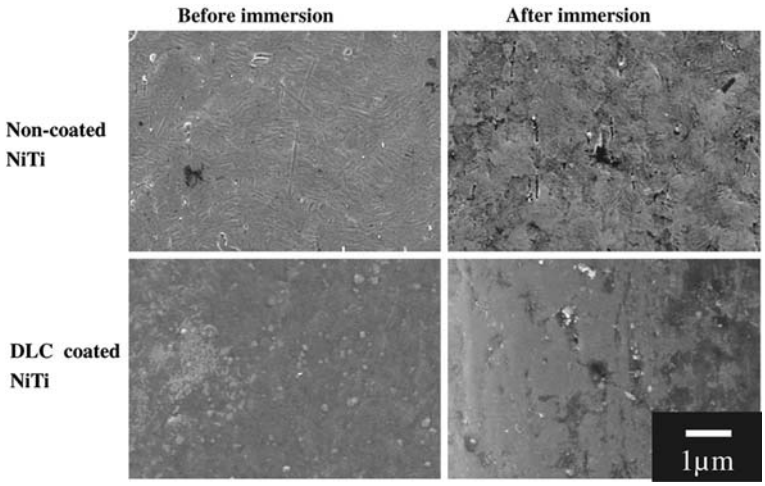

Fig. 4 SEM images of DLC-coated wire and non-coated wire before and after immersion in physiological saline for 6 months at 37 °C

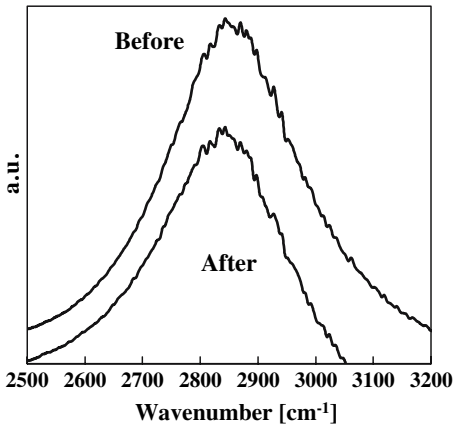

Fig. 5 Micro-FTIR spectra of DLC-coated archwires before and after immersion in physiological saline for 6 months

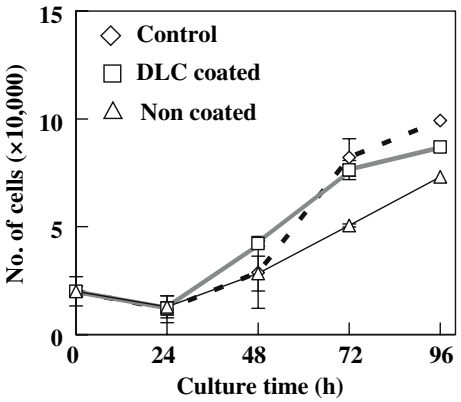

Fig. 6 Cell growth between growth Sa3 cells and the amount of Ni ion released

Table 1 C–H stretch absorption bands in FT-IR spectra

Configuration	Predicted frequency (cm^{-1})
sp^1CH	3,305
sp^2CH (arom.)	3,050
sp^2CH2 (olef.)	3,020
sp^2CH (olef.)	3,000
sp^3CH3 (asym.)	2,960
sp^2CH2 (olef.)	2,950
sp^3CH2 (asym.)	2,925
sp^3CH	2,915
sp^3CH3 (sym.)	2,870
sp^3CH2 (sym.)	2,855

Cytotoxicity test

Figure 6 shows the relationship between growth Sa3 cells and the amount of Ni ions released. From the cell growth curves, DLC film-coated wires showed higher cell growth rate in comparison with the non-coated wires. From SEM observation, there were no differences between DLC-coated and normal NiTi wires (Fig. 7).

Discussion

In this brushing test, a load of 35 g was applied using a toothbrush according to the load of actual daily brushing. This method is a more practical means of evaluating bond strength. After the test, DLC-coated wire showed the typical of the DLC Raman spectrum. This indicates that the DLC films have wear performance and good adhesion to the wire for the daily brushing.

For biomedical implants, it is important to know whether metal ions or wear particles were released from the implant surface [11]. This study indicates that DLC film is stable in the oral environment and protects NiTi archwires from corrosion. Because of its chemical inertness, DLC film not only acts as a physical barrier between the wire

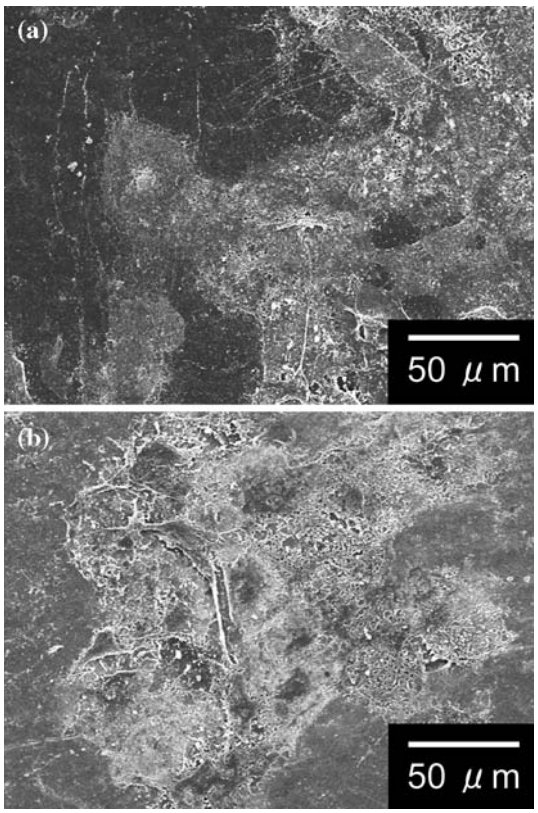

Fig. 7 SEM images of archwires after 96 h cell culture. (**a**) DLC-coated wire and (**b**) non-coated wire

and the corrosive environment, but also inhibits the release of Ni ions. Christine et al. reported that nickel is present in human tissue in quantities close to 0.1 ppm [12]. In this study, the concentration of nickel in physiological saline in which DLC-coated wires had been immersed for 6 months was less than 0.15 ppm, which is significantly below the tolerable upper intake level for the human body. These results correlate well with a previous report by Ozeki et al., who investigated the release of Ni ions from Ti-coated wires. Ni release from Ti-coated NiTi wires was inhibited to below 67.5 ppb for periods of 8 weeks [13]. In our study, we found that DLC films kept Ni release to below 50 ppb for 15 days. These results indicate that DLC films can improve the protective effect by 25.9% compared with Ti films. Huang et al. tested the anti-corrosive behavior of DLC films on stainless steel immersed in various solutions, and concluded that the non-crystalline structure of DLC remarkably improves the resistance of the steel substrate to pitting corrosion [4].

In the cell culture, the cell growth rate on the DLC-coated wires was higher than the non-coated NiTi wires (Fig. 6). There is significant difference between the DLC-coated wire and the non-coated wire after 72 and 96 h ($p < 0.01$). This clearly indicated that DLC films inhibit cytotoxicity effects. Nickel ions are cytotoxic, and induce pronounced reactions in animal experiments [14, 15]. Ohgoe et al. reported the growth rates of cells in the medium, adding the immersion fluid after the immersion test of NiTi wire in a physiological saline solution. They showed the cell growth rate was suppressed by the immersion fluid [16]. This indicates that the nickel ions have cytotoxicity.

However, we did not observe significant differences between DLC-coated wires and non-coated wires under SEM observation. Bogdanski et al. attempted to elucidate the biocompatibility of NiTi alloys with variable composition. They reported that NiTi alloys with a 50:50 Ni:Ti composition do not have cytotoxic effects on osteoblasts and fibroblasts cultured for 3 days, and the cells elongated on the alloy in microscopic observation. However, the cells NiTi alloy with a 60:50 NiTi composition showed rounded small shape, which indicates cytotoxicity [17]. These reports may indicate that the cell growth rate is more susceptible to the nickel ion concentration in comparison with the cell shape.

Conclusion

In brushing tests, DLC-coated wires showed excellent mechanical and adhesion properties. In immersion tests, the concentrations of Ni ions released from DLC-coated wires and non-coated wires after 6 months were 150 ppb and 900 ppb, respectively. In cytotoxicity tests, the cell growth rate on the DLC-coated wires was higher than the non-coated NiTi wires.

Acknowledgements The authors express their gratitude to Mr. Tuneo Toya for valuable technical assistance in this experiment. This study was supported by the Center for Research, Tokyo Denki University.

References

1. T. W. DUERIG, A. R. PELTON and D. STOCKEL, *Biomed. Mater. Eng.* **6** (1996) 255
2. K. TAKAMURA, K. HAYASHI, N. ISHINISHI and Y. SUGIOKA, *J. Biomed. Mater. Res.* **28** (1994) 583
3. K. OZEKI, T. YUHTA, H. AOKI andY. FUKUI, *Biomed. Mater. Eng.* **13** (2003) 271
4. G. F. HUANG, Z LINGPING, H. WEIQING, Z. LIHUA, L. SHAOLU and L. DEYI, *Diamond Relat. Mater.* **12** (2003) 1406
5. M. SUZUKI, T. WATANABE, A. TANAKA and K. KOGA, *Diamond Relat. Mater.* **12** (2003) 2061
6. F. PIAZZA, D. GRAMBOLE, D. SCHNEIDER, C. CASIRAGHI, A. C. FERRARI and J. ROBERTSON, *Diamond Relat. Mater.* **14** (2005) 994
7. M. K. FUNG, K. H. LAI, H. L. LAI, C. Y. CHAN, N. B. WONG, I. BELLO, C. S. LEE and S. T. LEE, *Diamond Relat. Mater.* **9** (2000) 815
8. A. GRILL, *Diamond Relat. Mater.* **12** (2003) 166

9. Y. OHGOE, S. TAKADA, K. HIRAKURI, K. TUCHIMOTO, A. HOMMA, T. MIYAMATSU, T. SAITOU, G. FRIEDBACHER, E. TATSUMI, Y. TAENAKA and Y. FUKUI, *Asaio J.* (2003) 701
10. B. DISCHLER, A. BUBENZER and P. KOIDL, *Solid State Commun.* **48** (1983) 105
11. M. F. CHEN, X. J. YANG, R. X. HU, Z. D. CUI and H. C. MAN, *Mater. Sci. Eng.* **C 24** (2004) 497
12. D. F. WILLIAMS, *Toxicology of implanted metals, Fundamental aspects of biocompatibility* (CRC Press, USA, 1981), p. 45
13. K. OZEKI, T. YUHTA, H. AOKI, T. ASAOKA, T. DAISAKU and Y. FUKUI, *Biomed. Mater. Eng.* **13** (2003) 355
14. L. TAN, R. A. DODD and W. C. CRONE, *Biomaterials* **24** (2003) 3931
15. M. BERGER-GORBET, B. BROXUP, C. RIVARD and L. H. YAHIA, *J. Biomed. Mater. Res.* **32** (1996) 243
16. Y. OHGOE, S. KOBAYASHI, K. OZEKI, H. AOKI, H. NAKAMORI, K. K. HIRAKURI and O. MIYASHITA, *Thin Solid Films* **497** (2006) 218
17. D. BOGDANSKI, M. KOLLER, D. MULLER, G. MUHR, M. BRAM, H. P. BUCHKREMER, D. STOVER, F. CHOI and M. EPPLE, *Biomaterials* **23** (2002) 4549