Enhancement of surface properties of biomaterials using plasma-based technologies

Paul K. Chu *

Department of Physics & Materials Science, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong

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Abstract

Development of new biomaterials typically takes a long time due to extensive tests and lengthy approval procedures. Plasma surface modification offers an exciting alternative by modifying selective surface mechanical and biological properties of conventional biomaterials to suit particular needs. Hence, materials that possess favorable bulk properties can have their surfaces redesigned to cater to biomedical applications. Plasma surface modification is a popular method to improve the multi-functionality, tribological and mechanical properties, as well as biocompatibility of artificial biomaterials and medical devices. Here, our recent research work on plasma modification of orthopedic nickel-titanium shape memory alloys and cardiovascular materials, namely diamond-like carbon is described. The shape memory effect and super-elasticity of NiTi alloys allow for a novel surgical technique for gradual correction of spinal deformity. However, out-diffusion of toxic Ni ions into human tissues is a health concern and plasma treatment is an excellent method to impede Ni leaching while the super-elastic properties of the bulk alloy can be retained. The two important requirements for cardiovascular materials such as those used in artificial heart valves, are that they must possess adequate surface mechanical properties and blood compatibility. Our recent experiments indicate that doping diamond-like carbon with elements such as nitrogen and phosphorus can enhance the biocompatibility of the materials.

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1. Introduction

Bio-integration of artificial biomaterials requires that the reactions occurring at the interface between the biomaterial surface and host tissues do not induce deleterious effects such as inflammation and irregular tissue response. Unfortunately, materials that possess favorable bulk properties such as strength frequently have inadequate surface biological properties such as blood compatibility. In this respect, surface modification plays an important role by providing a means to tailor selective surface properties without affecting the desirable bulk attributes of the materials [1]. Fig. 1 shows some of the common surface modification techniques. In particular, a number of plasma-based techniques are suitable for this purpose. They include radio-frequency (RF) glow discharge, electron cyclotron resonance (ECR) discharge, corona discharge, atmospheric plasma processes, sputtering, physical vapor deposition, chemical vapor deposition, plasma-assisted deposition, plasma implantation, plasma polymerization and grafting for polymeric surfaces, and plasma spraying [2]. Each technique has unique advantages and applications and the choice of the technique frequently depends on the reliability, reproducibility, and product yields [3], and plasma surface modification has become a hot research topic in biomaterials [4–6].

Among the various plasma treatment techniques, plasma immersion ion implantation and deposition (PIII&D) that was first introduced in the 1980s to circumvent the line-of-sight restriction of conventional beam-line ion implantation [7] offers advantages such as high efficiency, large area and batch processing, as well as small instrument footprint [8,9]. It has recently been applied to a variety of biomaterials research and development activities, such as orthopedic braces and materials [10–12], amorphous carbon films [13,14], hard tissue replacements [15,16], polymers [17–19], and titanium oxide [20]. In this paper, the recent developments in our laboratory pertaining to orthopedic
Nickel-titanium shape memory alloys and doped diamond-like carbon are described.

2. Plasma treatment of orthopedic nickel-titanium shape memory alloys

Stainless steels and titanium alloys are currently the most widely used metallic orthopedic materials. Stainless steels are the oldest and remain one of the most preferred materials for internal fixation devices because of their favorable mechanical properties, cost effectiveness and acceptable bio-compatibility [21]. Commercial titanium and titanium alloys are the best choice for dental and cementless orthopedic implants because they possess superior bio-compatibility and corrosion resistance as well as low modulus [22]. A new class of materials, nickel-titanium (NiTi) alloys, has recently attracted attention as orthopedic materials due to their unique super-elastic and shape memory effects [23–25]. While some studies have suggested that NiTi is compatible with living tissues [26,27], adverse effects have also been reported. For instance, it has been found that the osteogenesis process and osteonectin synthesis activity in NiTi alloys are unfavorable compared to stainless steels and titanium alloys [28]. Another study has reported severe cell death rate on NiTi alloys and the problem is believed to stem from the poor corrosion resistance and toxic substances released from the substrate [29]. The supernatant and corrosive products from the NiTi substrate may result in the death of smooth muscle cells, especially when the concentration of the leached nickel exceeds 9 ppm [30]. Other studies have shown that nickel leached from the alloys causes detrimental effects to humans, especially for nickel hyper-sensitive patients, resulting in strong allergic reactions [31,32]. Hence, the corrosion resistance of the materials must be enhanced before the materials can be more widely used clinically. Some researchers have implanted tantalum and oxygen using plasma techniques to improve the surface properties of NiTi alloys [33,34]. Our group has been

Fig. 1. Common surface modification techniques for biomaterials.

Fig. 2. Conventional surgical technique for correction of severe spinal deformity: (Left) X-ray of spine of a patient with scoliosis; (Middle) Surgeon straightening the spine with stainless steel rods; (Right) X-ray of spine after surgery.
investigating the enhancement of the corrosion and wear resistance as well as impediment of Ni out-diffusion from NiTi using plasma surface treatment. Our previous studies [35–38] have shown that the corrosion and wear resistance can be significantly improved by utilizing acetylene, nitrogen, and oxygen plasma immersion ion implantation (PIII). One advantage of PIII compared to conventional thin film deposition is the absence of an abrupt interface thereby reducing the problem associated with film delamination. In addition, its non-line-of-sight nature makes processing of medical implants with irregular geometries more effective. However, it should be mentioned that the treated region is typically quite thin, and so in applications in which extensive fretting and wear and tear occur, PIII may not be the most suitable technique. Nonetheless, in situations in which abrasive wear is not prevalent, PIII is an excellent surface modification technique. Here, we describe our latest results pertaining to the surface biological properties of PIII treated NiTi as well as the influence of mechanical stress on the materials.

Fig. 2 illustrates the conventional correction method for serious spinal deformity such as scoliosis. During surgery, the orthopedic surgeon uses stainless steel rods to straighten the spine of the patient. The degree of correction depends on the skills of the surgeon. A force which is too large can cause fracture as well as spinal cord damage, whereas too little force results in under-correction. Even in optimal cases, the degree of correction seldom exceeds 70% due to the visco-elasticity of biological tissues. Nickel-titanium alloys possess distinctive shape memory and super-elastic properties. That is to say, gradual correction under a constant force can take place inside the human body by using this type of materials in spinal surgery, thereby obviating the need for multiple corrective surgeries. In order to demonstrate the feasibility of this concept, we operated on a normal goat. As shown in Fig. 3, the spine of the goat was straight before surgery. We inserted a NiTi rod which was straight at 15 °C but became curved at the body temperature of 37 °C. Our results clearly show that the spine of the goat was gradually bent by the constant recovery force of the shape memory alloy rod thereby verifying the feasibility of the surgical procedures. In spite of the success, problems such as allergic reactions and impairment of cell proliferation stemming from nickel out-diffusion from NiTi when the materials are in direct contact with living tissues must still be tackled.

Our approach is to perform plasma implantation of carbon, nitrogen, or oxygen into the materials. The underlying principle is that Ti forms very stable bonds with these elements but nickel does not. Therefore, the preferential formation of Ti-O, Ti-C, or Ti-N bonds drives Ni away from the implanted surfaces [39]. Fig. 4 shows the XPS elemental depth profiles obtained from the carbon, nitrogen or oxygen plasma-implanted NiTi clearly showing depletion of Ni from the surface region. To further investigate Ni out-diffusion in a simulated biological environment, we employed soaking tests in simulated body fluid (SBF).
which has ionic concentrations similar to those of human blood plasma. After soaking the untreated control and PIII samples in SBF at 37 °C for 5 weeks, the solution was analyzed for nickel by inductively-coupled plasma mass spectrometry. As shown in Table 1, the PIII treatment reduces the leaching of Ni from the materials substantially. The surface mechanical properties such as hardness and Young’s modulus are also improved due to the formation of Ti-C, Ti-N, or Ti-C phases [40]. For instance, the surface nano-hardness improves by a factor of two.

To investigate the cyto-compatibility of the plasma-treated and untreated samples, osteoblasts isolated from calvarial bones of 2-day-old mice that ubiquitously expressed the enhanced green fluorescent protein (EGFP) were cultured in a Dulbecco’s Modified Eagle Medium (DMEM) (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (Biowest, France), antibiotics (100 U/ml of penicillin and 100 μg/ml of streptomycin), and 2 mM L-glutamine at 37 °C in an atmosphere of 5% CO2 and 95% air. Fig. 5 shows the degree of cell proliferation on the surfaces demonstrating that the plasma-implanted samples are cyto-compatible.

All these results have been obtained on unstressed samples, that is, samples that have not undergone shape change. In order to demonstrate the true efficacy of the technique, we have performed a bending test to: (1) determine whether the plasma-implanted NiTi alloys retain the shape recovery ability and (2) monitor the amount of leached Ni from the mechanically stressed NiTi. Our bending test follows the ASTM E855 3-point-bending protocol (MTS 858.02 Mini Bionix, USA) in a 37 °C water bath to simulate the temperature inside a human body. Our results show that if the temperature during PIII is low (∼150 °C), the rod undergoes shape recovery similar to the untreated NiTi control. However, if the sample temperature exceeds 200 °C during PIII, the rod loses the shape recovery properties. Therefore, proper control of the sample temperature is extremely important. Our SBF immersion test confirms that the properly plasma-treated sample retains very good surface barrier properties as the amount of Ni leached from the N-PIII treated, bent, and recovered sample is less than 20% of that leached from the untreated control sample. Hence, our results unequivocally demonstrate that the PIII treated samples retain

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ni concentration (ppb)</th>
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<tbody>
<tr>
<td>Untreated Control</td>
<td>865</td>
</tr>
<tr>
<td>Carbon PIII Sample</td>
<td>29.4</td>
</tr>
<tr>
<td>Nitrogen PIII Sample</td>
<td>31.2</td>
</tr>
<tr>
<td>Oxygen PIII Sample</td>
<td>36.2</td>
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Table 1
Ni concentrations in simulated body fluids after immersion for 5 weeks, determined by inductively-coupled plasma mass spectrometry

Fig. 5. Degree of osteoblast proliferation on the untreated control and PIII treated samples examined by scanning electron microscopy (SEM) after 2 days of cell culturing: (A) Untreated NiTi; (B) Nitrogen PIII NiTi; (C) Carbon PIII NiTi; (D) Oxygen PIII NiTi.
3. Doped diamond-like carbon coatings

Another recent application of PIII&D is to enhance the blood compatibility of artificial cardiovascular materials used in medical transplants such as artificial heart valves. Low-temperature isotropic pyrolytic carbon (LTIC) is currently the most widely accepted material, but the materials are quite brittle and the blood compatibility is still not sufficient. As a consequence, patients with LTIC heart implants must take anti-coagulation medication, and there is a need to develop new artificial heart valve materials that possess better blood compatibility and mechanical durability. The candidates include TiN, Ti(Ta+5)O2, and diamond-like carbon (DLC) thin films [41–45]. In particular, diamond-like carbon (DLC) or amorphous carbon films are projected to be a potential biomedical material due to their chemical inertness, low coefficient of friction, high wear resistance, and moderate biocompatibility [46,47].

Some of the properties of DLC can be improved by incorporating impurity elements into the matrix [48–51]. Two impurity elements, namely nitrogen and phosphorus, are of particular interest as they impact the electronic characteristics as well as the biological properties of DLC. In our nitrogen doping experiments, the doped DLC films were synthesized by operating a carbon filtered cathodic arc source in concert with a nitrogen/argon plasma in an immersion configuration (Fig. 6). Different mixtures of nitrogen to argon gases were introduced into the plasma to synthesize a series of thin films. Our data show that an optimized ratio of nitrogen to argon is necessary to achieve superior surface properties [50]. The blood compatibility of the materials was evaluated utilizing in vitro platelet adhesion tests. The platelet rich plasma (PRP) was extracted from human blood by centrifuging, and the lighter substances including platelets were separated from the plasma during centrifuging. After immersion, rinsing, fixing, and critical point drying, the adherent platelets on sample surface were examined using optical microscopy and scanning electron microscopy.

![Fig. 6. Schematic of apparatus for the synthesis of nitrogen doped diamond-like carbon films.](image)

![Fig. 7. SEM micrographs showing the morphology and quantity adherent platelets on: (a) Nitrogen-doped DLC and (b) LTIC.](image)
(SEM). Fig. 7 displays the morphology and quantity of adherent platelets on the nitrogen-doped DLC and LTIC. In addition to a reduced number, most of adhered platelets on the N-doped DLC films are isolated and round and very little destruction can be observed. On the other hand, most of the platelets on the LTIC exhibit pseudopodium indicative of some extent of activation. Our data suggest that an optimal fraction of sp² bonding is desirable and that graphitization induced degradation of the wettability properties should be avoided [50].

Phosphorus is widely used as an n-type impurity in silicon and is a possible alternative to nitrogen in carbon [52]. It is also a biologically friendly element, especially in bone structures. Hence, we have also investigated the influence of phosphorus doping on the biological properties of DLC. In our experiments, we employed a phosphorus evaporation source connected to a plasma immersion ion implanter to produce phosphorus-doped DLC films without breaking vacuum [53,54]. Again, platelet adhesion and activation are used as the indicators of thrombosis. The platelet adhesion test shows significant differences in the behavior of platelet adhesion among different materials. Fig. 8 shows the amounts of adhered platelets on LTIC, undoped DLC control, as well as P-doped DLC films after 20 min incubation. An average of 39 contact-adherent platelets are observed on the P-doped DLC film, compared to 70 on the LTIC as a control. The highest number (89) of adhered platelets is found on the undoped DLC film. The adhered platelets on the P-doped DLC and LTIC are observed to be isolated and relatively round. In comparison, most of the adherent platelets on the undoped DLC film are in aggregation, exhibiting spreading pseudopodium [51]. Further analysis shows that the surface of P-doped DLC exhibits excellent wettability, suggesting that one of the reasons for the good hemocompatibility is that the P-doped DLC coating significantly minimizes the interactions with blood plasma proteins, giving rise to slight changes in the conformation of adsorbed plasma proteins and preferentially adsorbed albumin [51].

4. Conclusion

Plasma surface modification is a versatile technique and has many advantages in the field of biomaterials engineering. For example, individual surface biological properties can be altered without changing the desirable bulk properties of the biomaterials such as strength and inertness. In this paper, we describe the recent applications of plasma immersion ion implantation to the surface modification of NiTi orthopedic materials as well as the synthesis and biocompatibility of nitrogen and phosphorus doped diamond-like carbon films as blood contacting materials. Our results show that PIII produces an effective surface barrier to mitigate nickel out-diffusion, and using the proper conditions, the PIII treated NiTi rods retain the shape recovery properties. The doped DLC films are observed to exhibit superior blood compatibility compared to LTIC and undoped DLC.

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