Biomedical Applications of Plasma and Ion Beam Processing

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Surface modification using plasma and ion beam processing can significantly change the chemical and physical characteristics of biomaterial surfaces, such as their structure, composition, surface wettability, electrical properties, mechanical properties, biochemical properties, etc. This kind of surface processing, sometimes in combination with other techniques such as chemical or biochemical methods, can be used to form novel biomaterial surfaces that are anticoagulant, antibacterial, bioactive, biomimetic, wear resistant, and more. In this paper we describe the application of plasma and ion beam processing to the field of biomedical materials.

1. Introduction

By plasma and ion beam processing we mean material surface modification by ions of energy in the broad range $10^2$ to $10^6$ eV. In physical processing of this kind, energetic ion bombardment can form surfaces with new characteristics, such as by the introduction of functional groups or thin film deposition on the matrix surface. Such modification can in turn lead to significant changes in the chemical and physical characteristics of the surface, such as structure, composition, surface wettability, electric properties, mechanical properties, biochemical properties, etc. It is possible in this way to modify biomedical implants to achieve improved biological surface properties for clinical application.

Plasma and ion beam processing has been applied for improving the properties of biomedical devices for the past three decades. Almost all surface related characteristics and performance can be modified by this kind of surface processing or by a combination with other surface modification methods. Table 1 presents some examples of the application of plasma and ion beam processing.

Many plasma techniques have been used for biomaterial modification. Among these, the two most representative processes are plasma surface modification and plasma thin film deposition. Plasma surface modification for biomaterials generally includes plasma treatment followed by grafting reactions, plasma-grafting, co-polymerization, etc. Plasma thin film deposition includes plasma polymerization, inorganic or metallic plasma coating, plasma assisted chemical vapor deposition, plasma-assisted physical vapor deposition (PAPVD), dual plasma deposition processes, and more. Ion processing is normally carried out higher ion energy, in the range $10^2$ to $10^6$ eV. Ions can penetrate the material surface to a depth of from several tens of nm up to as much as about $10^3$ nm; the ions are buried in the material to directly change the composition and material structure (ion implantation) or to combine with other species to form thin films (such as ion beam assisted deposition or plasma immersion ion implantation and deposition). Detailed descriptions of these techniques can be found in the literature1–16). This paper will focus on the applications of plasma and ion beam processing to the biomaterials field.

2. Plasma and Ion Beam Surface Modification applied to Biomaterials

From a materials aspect, surface modification by plasma and ion beam processing for treating medical devices can be categorized in two branches, for treating polymers and treating metal as well as the inorganic matrix. Here we discuss plasma and ion beam surface modification as applied in these two biomaterial branches.

2.1 Polymer Surface Modification by Plasma and Ion Beam Processing

For polymer surface modification, research dealing with plasma and ion beam processing has addressed improvement in the biocompatibility of polymers to be utilized in vivo, ex vivo or in vitro.

2.1.1 Surface grafting by plasma processing
Poly (ethylene glycol)(PEG) is a highly water-soluble polymer. Its flexible long chains are often used to improve blood compatibility because this structure can influence the micro-kinetic environment of the biomaterial-blood interface, interfere with the adsorption of plasma protein, and prohibit the formation of thrombi. Grafting is an effective method for modification of the polymer surface, and PEG is usually grafted on the polymer surface to resist protein and platelet adhesion17–19). The hydrophilicity of a PEG molecular grafted surface is dependent on the molecular weight of the PEG, and thus the protein adsorption decreases with increasing PEG chain length. Reduced coagulation activation, blood platelet adhesion, and complement activation have been found for such modified surfaces20).

Wang et al.21) treated poly (ethylene terephthalate) (PET), used in artificial heart valve sewing rings, by plasma discharge and grafted with different molecular
### Table 1  Biomaterial applications of plasma surface modification (www.astp.com/PDFs/PS_bimed.pdf)

<table>
<thead>
<tr>
<th>Application</th>
<th>Devices</th>
<th>Materials</th>
<th>Purposes of plasma and ion beam surface modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosensor</td>
<td>sensor membranes</td>
<td>PC, Cellulose, Cuprophane, PP, PS</td>
<td>Immobilization of biomolecules, non-fouling surfaces</td>
</tr>
<tr>
<td>Bioseparation</td>
<td>separation membranes</td>
<td>PP, cellulose derivatives, PSF</td>
<td>Anti-fouling surfaces</td>
</tr>
<tr>
<td></td>
<td>hemodialysis membranes</td>
<td></td>
<td>wetability enhancement</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Vascular grafts catheters</td>
<td>PET, PTFE PE, SIR, PVC, PU</td>
<td>Improved biocompatibility (endothelialization)</td>
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<td></td>
<td></td>
<td></td>
<td>wetability enhancement</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Improved biocompatibility</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lubricious coatings</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>reduced friction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>anti-microbial coatings</td>
</tr>
<tr>
<td>Dental</td>
<td>dental implants</td>
<td>Ti alloys</td>
<td>enhanced cell growth</td>
</tr>
<tr>
<td>Fundamental research</td>
<td>Polymers, Metals, Glass, Ceramics, Composites</td>
<td>Biological interactions with plasma gas discharge-modified surfaces</td>
<td></td>
</tr>
<tr>
<td>Ophthalmological</td>
<td>contact lenses</td>
<td>PMMA, SIR</td>
<td>wettability enhancement</td>
</tr>
<tr>
<td></td>
<td>intraocular lenses</td>
<td>PMMA, SIR</td>
<td>improved biocompatibility</td>
</tr>
<tr>
<td></td>
<td>artificial corneas</td>
<td>PVA, PHHEMA</td>
<td>anti-microbial coatings</td>
</tr>
<tr>
<td>Orthopedic</td>
<td>metal implants</td>
<td>Ti-Ni, Co-Cr alloys</td>
<td>surface cleaning (etching)</td>
</tr>
<tr>
<td></td>
<td>joints</td>
<td>UHMWPE</td>
<td>enhanced cell adhesion/growth</td>
</tr>
<tr>
<td></td>
<td>ligaments</td>
<td>PET</td>
<td>improved biocompatibility</td>
</tr>
<tr>
<td></td>
<td>bone plates</td>
<td>PGA, PLA</td>
<td>enhanced bone cement adhesion</td>
</tr>
<tr>
<td>Pharmaceutical</td>
<td>drug controlled release devices</td>
<td>SIR, hydrogel, PGA, PLA</td>
<td>reduced molecular diffusion</td>
</tr>
<tr>
<td>Tissue culturing</td>
<td>tissue culture dishes</td>
<td>PS, PET</td>
<td>controlled degradation for drug release</td>
</tr>
<tr>
<td>Others</td>
<td>general devices</td>
<td></td>
<td>enhanced cell adhesion/growth</td>
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<td></td>
<td></td>
<td>wettability enhancement</td>
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<td>surface cleaning (etching)</td>
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<td>adhesion promotion</td>
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Abbreviation of polymers: PET: poly(ethylene terephthalate); PTFE: polytetrafluoroethylene; PE: polyethylene; SIR: silicone rubber; PVC: polyvinyl chloride; PU: polyurethane; PMMA: polymethyl methacrylate; UHMWPE: ultra high molecular weight PE; PGA: poly(glycolic acid); PLA: poly(lactic acid); PS: polystyrene; PC: polycarbonate; PP: polypropylene; PSF: polysulfone.

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water-soluble PEG. The PET films were first treated in oxygen plasma and then dipped in solutions of PEG with molecular weights of 200, 1000, 6000 and 10,000 for 6 h. The samples were dried in a clean room and treated a second time using an argon plasma. The interaction between the surface-modified PETs and blood components was investigated to evaluate the blood compatibility of the samples.

Activated partial thromboplastin time (APTT) of the PET-PEG was significantly longer than for the untreated PET, as shown in Fig. 1. Scanning electron microscopy (SEM) and optical microscopy indicated that adhered, aggregated and morphologically changed platelets were significantly reduced by grafting PEG chains on the PET films, as show in Fig. 2. In vitro blood compatibility tests showed that the blood compatibility of PET grafted...
with PEG was related to the molecular weight of the PEG. The best blood compatibility was achieved when the grafted PEG molecular weight was 6000. The hemocompatibility improvement can be explained by the low interfacial free energy, sterically hindered effects of PEG chains, and maintaining of the normal conformation.

Immobilization of biomolecules on polymer surfaces using plasma processes is another attractive strategy. It is known that heparin and heparin-like molecules, collagen, albumin, Arg-Gly-Asp peptide (RGD) and other molecules of biological origin, confer on the surface a bioactive or biomimetic nature. However, standard polymers exhibit a high degree of chemical inactivity. There are few functional groups on the surface that can bind with biomolecules. Biomolecules may be adsorbed on the surface due to van-der-Waals dispersion forces, hydrogen bonding, or acid-base interactions. These kinds of surfaces can be varied reproducibly, and may affect the effectiveness of the function of the surface. Therefore functionalization of the surface is of particular importance for biomolecule immobilization. Chemical functionalities such as amino, hydroxyl, carboxyl, and epoxy groups are known to be effective in covalent coupling of proteins and signal molecules. Therefore functional groups can be seen to control the immobilization of biomolecules.

Meyer-Plath et al.25 reviewed the state of surface functionalization using nitrogen plasma processes. In contrast to most other functional groups, the protonated amino group introduces a localized positive charge to the polymer and may, in aqueous solution at physiological pH values, primarily attract negatively charged biomolecules. In addition, due to their good chemical reactivity, amino groups are widely used in biochemistry for covalent coupling of proteins in aqueous environments. This is why amino groups are widely believed to be efficient in immobilizing biomolecules and in promoting cell adhesion. Many types of polymers have been successfully equipped with amino groups by various low-pressure plasma processes. Their biomedical applications have been studied by many authors 25-28.

Terlingen et al.25 carried out a study on introducing amine groups on poly (ethylene) (PE) surfaces. They preadsorbed PE surfaces with decylamine hydrochloride and subsequently treated with an argon plasma. It was shown by XPS (X-ray Photoelectron Spectroscopy) that approximately half of the preadsorbed (mono) layer was immobilized and that a substantial part (60–70%) of the incorporated nitrogen containing groups were amine groups. The availability of the surface amine groups for reactions was investigated by applying a gas phase reaction with 4-trifluoromethylbenzaldehyde and by a reductive methylation reaction in aqueous solution with formaldehyde. A maximal number of reactive amine groups was found after a plasma treatment time of 2 s. The reductive methylation reaction was used to estimate the surface concentration of amine groups resulting in a typical surface concentration of $1 \times 10^{-6}$ mol/m² after a plasma treatment time of 2 s.

Keen et al.29 applied radio frequency ammonia plasma treatment to introduce amine functionalities on the surface of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) films and by wet ethylenediamine treatment. The relative amount of amine functionalities introduced onto the PHBV surface was determined by exposing the treated films to the vapor of trifluoromethylbenzaldehyde (TFBA) prior to XPS analysis. The greatest amount of amino groups on the PHBV surface was obtained by use of ammonia plasma for short treatment times of 5 and 10 s, but no effect of plasma power within the range of 2.5–20 W was observed. Ethylenediamine treatment yielded fewer surface amino groups, and in addition an increase in crystallinity as well as degradation of PHBV was evident from Fourier transform infrared spectroscopy. Raman maps showed that the coverage of amino groups on the PHBV surfaces was patchy with large areas having no amine functionalities.

Hadjizadeh et al.27 reported the fabrication of bioactive polymer fibers on which signaling molecules can control and direct cell responses. To encourage and control directional biological responses, Gly-Arg-Gly-Asp-Ser (GRGDS) peptides were immobilized on the surface of 100 micron diameter PET fibers (monofilaments). PET fiber surfaces were first coated with a thin polymer interfacial bonding layer bearing amine groups by plasma polymerization. Carboxy-methyl-dextran (CMD) was covalently grafted onto the surface amine groups using water-soluble carboxidime chemistry. GRGDS were covalently immobilized onto CMD-coated fiber surfaces. X-ray photoelectron spectroscopy (XPS) analysis enabled characterization of the multilayer fabrication steps. Human umbilical vein endothelial cells were seeded and grown on fibers to investigate cell patterning behavior (i.e., adhesion, spreading, cytoskeleton organization, and cell orientation). Cell adhesion was reduced on CMD-coated fibers, whereas amine- and GRGDS-coated fibers promoted cell adhesion and spreading. Cell adhesion was enhanced as the GRGDS concentration increased. Epifluorescence microscopic visualization of cells on RGD-coated substrates showed well-defined stress fibers and sharp spots of vinculin, typical of focal adhesions. In comparison to plasticware commonly used in cell cultures, fiber curvature promoted cell orientation along the fiber axis.

Baquiey et al.28 described how the surface of poly (vinylidene difluoride) (PVDF) can be activated with swift heavy ions and investigated the use of a radio-fre-
frequency glow discharge (rfGD) on expanded polytetrafluoroethylene (e-PTFE). With regard to grafting of peptide, a multi-step approach can be carried out as shown schematically in Fig. 3. It has been shown that the plasma treatment effectively alters the surface of the e-PTFE. The oxygen content rises whereas the ratio of fluorine to total carbon atomic concentrations (F/C) drops. At the same time, plasma treatment of e-PTFE results in the activation and subsequent surface functionalization, and the relevant cell attachment results are exhibited in Fig. 4.

PET is a typical versatile biomaterial mainly used in artificial heart valve sewing cuffs and vascular grafts. Infection from implanted medical devices is a life threatening complication, leading to significant morbidity and mortality. In particular, the incidence of prosthetic valve endocarditis (PVE) is about 2%–3% in patients undergoing valve replacement, with staphylococcus epidermidis (SE) accounting for about 30% overall of these infections. The bacterial adhesion to the biomaterial substrate is the first event in a series of both host and organismic reactions that leads to PVE. This adhesion is mediated by physicochemical interactions between the bacteria and substrate. As a result, a significant number of studies on improving the antibacterial adhesion of polymer have focused on surface modification. Li et al. investigated the influence of chitosan-immobilization on bacterial adhesion on PET films. They washed and dried PET films and then exposed them to an argon plasma glow discharge. The grafting polymerization of acrylic acid (AA) was induced to introduce carboxylic acid groups onto PET (PET–AA) assisting with ultraviolet radiation. After that, PET–AAs were immersed into the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide solution at 4°C for 4 h to activate the carboxylic acid groups on PET–AA surface. Finally, the activated PET–AA was immersed in acetic acid solution of chitosan and the chitosan-immobilized PET films were obtained after the chemical reactions between chitosan and PET–AA. The bacterial adhesion on PET surface was evaluated by bacteria plate counting in vitro and SEM as shown in Fig. 5. It was shown that antibacterial characteristics were brought about by the immobilization of chitosan molecules on the PET surface. The amount of bacteria adhered to the chitosan-immobilized PET surface is much less than for the untreated PET surface.

**2.1.2 Surface polymerization**

Krishna et al. report a novel method for surface grafting a polymeric phospholipid system containing an acryloyl end group (1stearoyl-2-[12-(acryloyloxy)dodecanoyl]-sn-glycero-3-phosphocholine) onto medical grade silicone catheters. The surface of silicone catheters was functionalized in a sequence of steps: plasma polymerization of allyl alcohol on the catheter surface, grafting acryloyl moieties and in situ polymerization of the pre-assembled acryloyl terminated phospholipids on the acryloyl functionalized catheter surface. The surface morphological changes were analyzed by SEM and atomic force microscopy (AFM). A sharp decrease in water contact angle, and the appearance of an N1s peak in XPS analysis indicated successful monolayer grafting.

![Fig. 3 Schematic of peptide immobilization](image)

![Fig. 4 Cell attachment onto the modified surface of peptide and control sample as reflected by percent of absorbance with respect to the control](image)

![Fig. 5 Scanning electron micrographs of PET-K (untreated) (a), and PET-CHI (chitosan immobilized) (b); scale bar represents 10 μm.](image)
of the phospholipid. In platelet adhesion tests performed using platelets isolated from rabbit plasma, the phospholipid grafted surface showed fewer adhered platelets, without emerging pseudopodes or aggregation. However, ungrafted catheter surface showed a large number of platelets in extensively spread and aggregated states. Thus this modified phospholipid system and its simple grafting technique was very effective with regard to suppressing in vitro platelet adhesion on the silicone catheter surface.

Elvira et al. performed plasma and chemical induced graft polymerization of acrylic monomers on starch-based biomaterials to improve cell adhesion and proliferation on the surface of the polymers, in order to evaluate their properties for bone tissue engineering scaffolding applications. Plasma and chemical surface activation was aimed at inducing the polymerization of acrylic polar monomers, being carried out by applying a radio frequency plasma and exposing the samples to a mixture of Ar/O2, or by immersion in a H2O2/(NH4)2S2O8 solution with UV radiation. Both procedures were followed by the graft polymerization of the corresponding monomers. Polymer grafting was analyzed by Fourier transform infrared spectroscopy (FTIR) and XPS and by contact angle measurements. Properties such as mechanical performance, swelling degree, and degradation behavior, as well as bioactivity, were studied and compared for the different activation methods. Cell adhesion and proliferation tests performed using goat bone marrow cells showed a remarkable improvement with respect to original non-surface modified starch-based biomaterials.

Lahann researched the improvement of haemocompatibility of metal stents by polymer coating. Implantation of intracoronary metal stents is an alternative to open heart surgery in treating arterial diseases causing restricted blood flow. In spite of the advances in implantation and in spite of the excellent mechanical properties of metal stents, there are still limitations because of the thrombogenicity of the metal. They synthesized a coating on the stents with an ultrathin polymer layer by polymerization of 2-chloroparacyclophan. In a second step of surface modification the poly (2-chloroparaxylylene) layer was modified by treatment with a sulfur dioxide plasma in order to obtain a more hydrophilic surface with new functional groups. The results demonstrated the stable polymer coating of the stents and the improvement of haemocompatibility after treatment with sulfur dioxide plasma. Platelet adhesion was decreased from 85 % for the metal surface to 20% for the coated and sulfur-dioxide-plasma treated surface.

2.1.3 Polymer surface modification by ion beam processing

In beam line ion implantation processing, the ion dose can be precisely controlled. It is possible to implant the required ions with expected doses into an expected position of the surface layer. Implantation and deposition processing can be performed at ambient temperature. It can be used to treat metal, ceramics and polymer materials. In the investigation of implantation of silicone with Na+, H2+, He+, O+, O2+, N+, N2+, Ar+, K+, Kr+ and other ions, it is found that platelet adhesion decreases significantly on surfaces implanted with O2+ at energy of 150 keV and dose 1-2 x 1017 ions/cm2. It is considered that the formation of >C=O groups and an amorphous carbon film on the surface is the reason for the modification. Moreover, experiments show that ion implantation can effectively alter the surface polarity and wettability of polypropylene and polystyrene and improve anticoagulation and anticalcification, increase EC adhesion, etc., of the materials. Beam techniques have also been used for endothelial cell seeding processes. He+ ions of energy 150 keV and dose 1 x 1017 ions/cm2 were implanted into collagen coated ePTFE tubes to improve endothelial cell binding on the surface. In vivo tests of the EC bound tubes implanted into dog artery and vein for 120 days showed no thrombogenicity, while the untreated ePTFE tubes were occluded 3 days after implantation.

Compared with beam line ion implantation processing, plasma immersion ion implantation, with a non-line-of-sight feature and higher efficiency, has attracted greater attention in the biomedical field.

A typical example of the application of plasma immersion ion implantation to polymer surface modification is the treatment of PET for improving its biocompatibility. Wang and Huang et al. conducted acetylene (C2H2) plasma immersion ion implantation (PIII) into PET. It was found that amorphous hydrogenated carbon (a-C:H) films were formed on the PET. Investigation of blood compatibility of the modified PET by platelet adhesion and activation behavior showed that the adhered platelets on treated PET at 0.5 Pa and 1.0 Pa working pressure are about 32% and 55%, respectively, of that for the untreated PET surface. The platelets are observed to be isolated and round on carbon films deposited at 0.5 Pa, as shown in Fig. 6, indicating that fewer platelets are activated on the amorphous carbon films. These results

| Table 2 Instrumental parameters for the synthesis of a-C: H films |
|-----------------|-------------|-------------|-------------|---------|--------|-------|
| Sample          | Working pressure (Pa) | Bias voltage (kV) | Pulse frequency (Hz) | Pulse width (As) | RF power (W) | Time (min) | Thickness (nm) |
| PET-C1          | 0.5         | 5           | 100         | 20       | 300    | 40     | 102         |
| PET-C2          | 1.0         | 5           | 100         | 20       | 300    | 40     | 195         |
| PET-C3          | 2.0         | 5           | 100         | 20       | 300    | 40     | 245         |

Fig. 6 Morphology of adherent platelets on (a) PET (untreated) and (b) PET-C1.
thus show that amorphous carbon films deposited on PET by C2H2 PIII suppress platelet adhesion and activation, and the extent of the improvement is related to the structure of the carbon films.

More interesting is that this treatment is not only effective for improving blood compatibility of PET, but also conveys bacterial adhesion limitation properties\(^{(39)}\). They investigated the antibacterial adhesion behavior on PET treated by plasma immersion ion implantation and deposition (PIII–D) using acetylene (C\(_2\)H\(_2\)) at different working pressures. The ability of Staphylococcus aureus (SA) and Staphylococcus epidermidis (SE) to adhere to PET was quantitatively determined by plate counting and gamma-ray counting of the \(^{125}\)I-labeled bacteria \textit{in vifro}. The adhesion efficiency of SA on the a-C: H film deposited at 0.5 Pa working pressure was about 16\% of that on the untreated PET surface, and the adhered bacterial concentration of SE on the carbon film deposited at 1.0 Pa was about 1/6 that of the PET surface as shown in Fig. 7. Bacterial adhesion on a-C: H films is influenced by the structure and chemical bonds of the material. They explained the reduction in bacterial adhesion by the free energy of adhesion (\(\Delta F_{\text{adh}}\)), which predicts whether microbial adhesion is energetically favorable (\(\Delta F_{\text{adh}} < 0\)) or not (\(\Delta F_{\text{adh}} > 0\)). These results show that bacterial adhesion is energetically unfavorable on a-C: H films deposited at 0.5 and 1.0 Pa, and this study suggests one possible method to repel bacteria from polymeric surfaces.

2.2 Plasma and Ion Beam Surface Modification applied to Metallic and Inorganic Biomaterials

The inertness of the surfaces of metal and inorganic materials is regarded as being so high that it is difficult to form functional groups on the surface by using plasma treatment or grafting. Therefore most modification of metal and inorganic surfaces is done by depositing films or coatings.

2.2.1 Surface thin film deposition on metal and inorganic biomaterials

Many thin films have been explored for their value in the field of biomaterials, such as TiN, SiN\(_2\), diamond-like carbon (DLC), TiC, SiC, Al\(_2\)O\(_3\), ZrO\(_2\), TiO\(_2\), etc. This section describes three widely investigated films or coatings:

(a). Carbon based films

DLC and CN are regarded as important candidate thin films for application to biomedical implants. Because they possess excellent mechanical properties, a high degree of inertness, and consist only of carbon, hydrogen and nitrogen, they are biologically compatible. Hard DLC and CN coatings can be considered for (a) surgical instruments, (b) implanted fittings such as infusion devices and connecting parts, and (c) semi-permanent components such as heart-valves, replacement joints, and ophthalmic devices. DLC films can be deposited by a variety of plasma methods, including hot filament, microwave, rf or dc PECVD, laser plasma deposition, and graphite/metal PIII–D\(^{(40),(41)}\). Amorphous CN coatings have also been prepared in an attempt to synthesize C\(_n\)N\(_m\) using several plasma-based approaches including plasma decomposition of methane and nitrogen\(^{(42)}\) and rf sputtering of a graphite target in an argon/nitrogen plasma\(^{(43)}\). There is no indication that the DLC coating is dissolved or is toxic to cells. No delayed attachment is observed when HEK 293 cells are freshly seeded onto DLC-coated dishes compared to cells cultured in plastic. These results demonstrate that DLC coatings express no toxicity to cultured human ML–1 and HEK 293 cells\(^{(44)}\).

Due to the excellent wear resistance of DLC films, an active area of DLC film application in biomaterials is for coating hip joints.

Huang et al\(^{(45)}\) investigated the wear resistance of Ti6Al4V coated with DLC. According to wear trace and friction coefficient analyses, an increase of several orders of magnitude in wear resistance of the Ti6Al4V can be achieved by DLC coating (see Fig. 8). This offers great potential for using DLC films to improve the durability of biomedical devices. The DLC coated head of a hip joint is shown in Fig. 9.

Dorner-Reisel et al.\(^{(45)}\) performed wear testing for 5 million cycles under tribological loading in a knee joint simulator, and found that diamond-like carbon coatings with optimized structure and adhesion remain unchanged on the surface of Co28Cr6Mo femoral segments. The tribological loading was carried out in bovine serum solution with DLC-coated Co28Cr6Mo femurs articulated against ultra-high molecular weight polyethylene (UHMWPE) inlays. The wear resistance according to ISOyDIS 14 243–1 is evaluated by the weight loss of the UHMWPE inlays. After wear testing, diamond-like carbon coatings with optimized structure and adhesion
remain unchanged on the surface of the Co28Cr6Mo femoral segments. Furthermore, the wear of the UHMWPE inlays is affected by the DLC thickness. A 4.5 \mu m thick DLC coating led to a strong decrease of wear resistance of the UHMWPE counter-face. The wear of the UHMWPE inlay increases with increasing DLC thickness. The 4.5 \mu m thick DLC coating causes crucial increased wear of the polymer. A possible reason may be changes in heat transfer. The heat transfer may be reduced with DLC coating thickness and the lower fraction of defects, like pinholes, in thicker DLC coatings. In comparison with uncoated Co28Cr6Mo femoral components, the release of metallic wear products is reduced, due to the coverage of the metal alloy with DLC.

Testing in a knee wear simulator using distilled water as lubricant, Onate et al.\textsuperscript{46} obtained a decrease of a factor of five in wear of the UHMWPE by coating the cobalt-chromium counter-face with DLC. DLC was deposited using CC800–8 CemeCon PVD equipment involving initial deposition by magnetron sputtering. The thickness of the coatings was in the range 3–4 \mu m. The lowest and almost negligible polyethylene material transfer takes place on DLC and Al2O3 ceramic materials. The application of DLC coatings and ion implantation on Co-Cr alloy are very valid options for manufacturing knee implants with greater longevities, by reducing significantly the generation of UHMWPE debris and hence the risk of osteolysis.

However, other researchers have reported that the wear resistance of hip joints is not significantly improved by DLC coating. Saikko et al.\textsuperscript{47} performed deposition of DLC films with thickness of 3 \mu m on Co-Cr heads. Wear tests were done using a new three-station BRM hip wear simulator. The lubricant was prepared with triple 0.1 \mu m sterile filtered, low-protein, low-endotoxin HyClone Alpha Calf Fraction serum, catalogue number SH30076.03, diluted 1:1 with Millipore distilled water. The average wear rates of the cups against alumina and CoCr heads were 48 and 56 mg per 1 million cycles, respectively, in order-of-magnitude agreement with clinical observations, while the average wear rate against DLC was 58 mg per 1 million cycles. As a counterface for polyethylene, DLC did not markedly differ from alu-

mina and CoCr. In the authors’ earlier BRM simulator studies using a single-station prototype, the wear rates of similar cups were 22 and 23 mg per 1 million cycles against alumina and CoCr, respectively. In that study, Sigma B–2771 adult bovine serum was used as lubricant, diluted 1:2 with distilled water, resulting in a protein concentration of 24 mg/ml. Sodium azide was added to the lubricant (0.2 percent), and the lubricant change interval was as long as 1 million cycles. Their experiments revealed that the differences in lubrication conditions may explain the large differences in wear between these two studies.

The above completely different results show that the full story about the behavior of DLC performance as a hip joint coating is not yet understood very clearly. There are many factors that can affect the tribological behavior of the DLC-UHMWPE couple, such as material factors including the content of sp\textsuperscript{2} and sp\textsuperscript{3} bonds in the DLC film, composition, adhesion strength of the DLC film to the matrix, film thickness, surface roughness, internal stress in the film, degree of cross linking, degree of crystallinity, molecular weight of the UHMWPE, as well as lubrication conditions, loading state, contact state of the counterparts, fabrication accuracy of the wear couple, etc. Further systematic research is needed.

Improving the blood compatibility of implants using DLC films is an interesting topic. Dion et al.\textsuperscript{48} have determined the plasma protein adhesion on silicone elastomer and DLC coated Ti-6Al-4V, and found that more albumin adheres to the DLC coating than to the medical grade elastomer, and there is more fibrinogen than on the silicone elastomer.

Huang et al.\textsuperscript{16} found that the relationship between the DLC film characteristics and blood compatibility is very complicated. Their research shows that it is possible to modify the blood compatibility of DLC films by changing bonding state, doping of the film, and other. Hydrogenated DLC films were synthesized using acetylene gas plasma immersion ion implantation and deposition. In the investigation, a DLC film prepared at lower bias voltage, such as −75 V, showed low platelet adhesion and activation, similar to LTIC and better than stainless steel. When prepared at high bias, such as −900 V, platelet adhesion and activation properties of the DLC film are not good. It seems that a higher sp\textsuperscript{2}/sp\textsuperscript{3} bonding ratio may contribute to the blood compatibility of DLC. However, as the DLC film was doped with nitrogen, the platelet adhesion behavior was improved significantly, and was even superior to that of LTIC even when the sp\textsuperscript{2}/sp\textsuperscript{3} bonding ratio was decreased.

Gutensohn et al.\textsuperscript{49} deposited DLC films on stent surfaces, and found that such films can serve as a barrier against elution of heavy metal ions from stainless steel to surrounding tissue and blood and improved biocompatibility compared to 316 L stainless steel surfaces. The activation of polymorphonuclear leukocytes and platelets was significantly reduced on DLC, and inflammatory processes were significantly reduced as well. The thrombocyte activity markers CD 62p and CD 63 were reduced.
for diamond-like carbon coated stents compared with uncoated stents. Experiments on total occlusion (the stent was expanded inside a plastic tube through which the blood was circulating until total occlusion by thrombus) showed a lower thrombogenicity of DLC in comparison with stainless steel. The adherence of human umbilical endothelial cells was also improved on DLC in comparison to stainless steel.

DLC coated heart valves and stents are already commercially available or at a development stage. The Cardio Carbon company offers DLC-coated titanium implants (“Angelini Laminato” mechanical heart valves and “Angelini Valvuloplasty” rings); Sorin Biomedica produces heart valves and stents coated with CarbofilMTM; the PHYTIS company sells DLC coated stents50).

Another kind of carbon thin film coating, called nanocrystalline diamond, has also been investigated for biomedical implant purposes51).

Jozwik and Karczemka52) reported the durability of a nanocrystalline diamond (NCD) coated artificial heart valve ring. They performed fatigue testing of a Ti6Al4V heart valve coated with NCD and having a Derlin disc at 1000 cycles per minute and a testing period of 14 months. Before and after the mechanical fatigue experiments, the surface of the valve ring was examined by Scanning Electron Microscopy (SEM) and by Raman Spectroscopy. Comparative studies before and after mechanical fatigue experiments showed that the surface after the experiments is in good condition, still tight, and the range of the NCD layer thickness is the same as before the operation. Raman spectra indicated that the NCD coating still covered the full ring surface.

(b). Titanium oxide films

It is believed that the biocompatibility of titanium derives from characteristics of the naturally formed titanium oxide film on its surface53). However, this naturally formed oxide layer is very thin (about 10 nm) and has a high density of defects. Better biocompatibility has been reported by improving the quality of the titanium oxide film54–59).

Huang et al.60) found that non-stochiometric and tantalum-doped titanium oxide films formed by plasma immersion ion implantation and deposition processing. Huang et al. deposited titanium oxide films and carried out systematic research on the relationships among structure characteristics, different composition, surface physical-chemical properties (such as electrical, semiconducting, and surface tension) of the titanium oxide films and biological responses of blood on the film surface (protein adsorption and denaturation, platelet adhesion and activation, clotting factor activating, in vivo implantation, etc.) It was found that the surface tension and the semiconducting characteristics are both important factors affecting blood compatibility of Ti-O films. A better platelet adhesion state can be reached as the interface tension between the Ti-O film and the biological substance such as protein is decreased61).

However, the semiconducting nature of Ti-O films is the most decisive factor in determining the blood compatibility behavior of the material. A significantly high level of blood compatibility can be achieved by forming a sub-stoichiometric Ti-O film and doping with P, H and Ta. Such a film possesses the characteristics of an n-type semiconductor60,62). In contrast, films doped with Al have p-type semiconductor characteristics and show very poor blood compatibility. A detailed investigation of the conformation state of fibrinogen absorbed on the Ti-O film surface shows that the relative quantity of denatured fibrinogen on n-type Ti-O film decreases dramatically compared with stoichiometric TiO2 films. It is believed that n-type Ti-O films have the ability to prevent charges from transferring from fibrinogen into the material and thereby prevent the protein from denaturation63). Fig. 10 shows the interaction of fibrinogen with an n-type semiconductor. Electron density in the conduction band is higher, which could play a role in compensating the local state in the band-gap and the surface state of the semiconductor. It is difficult for charges to transport from the valence band of fibrinogen to the material. On the other hand, a p-type semiconductor with a high density of holes has a low energy level. Electrons in fibrinogen can be transported into the material, resulting in a change of the configuration of fibrinogen.

Fig. 11 shows the morphology of platelets adherent to a P doped TiO2 film and an Al doped TiO2 film. The P doped TiO2 film is an n-type semiconductor, and the platelet adhesion state is significantly better than for the Al doped TiO2 film which possesses a p-type semiconductor nature.

Ti-O films have also been applied for the surface modification of stents. Huang et al.64) reported work on surface coating of Ti-O/Ti-N complex films on coronary stents by means of plasma immersion ion implantation/deposition processing. The deformation behavior of the Ti-O/Ti-N coated stainless steel stents was investigated. In vivo investigation of the anticoagulation behavior of Ti-O coated coronary stents was also performed. The results of mechanical characterization of the Ti-O/Ti-N coated stents showed that the film has a
strong binding strength, and to some extent the ability to withstand plastic deformation. The biological response behavior of the coated stent surface was significantly different from the uncoated. As shown in Fig. 12 and Fig. 13, implantation of stents into rabbit ventral aorta shows no thrombus formation on the surfaces of the TiO coated stents, although serious coagulation had occurred on the surfaces of unmodified stents over a period of 4 weeks under conditions with no anticoagulant.

(c). Calcium phosphate compound films

Calcium phosphate apatite possesses good properties as a biomaterial for bone repair, augmentation, substitution, and surface coating. Coating the surfaces of dental and orthopedic materials with biocompatible calcium phosphate apatite can elicit favorable biological and chemical responses, and this allows researchers to mimic the reactions occurring in natural calcified tissue without compromising the bulk properties of the material such as durability and inertness. Hydroxyapatite (HA), which has many crystallographic features similar to those of the natural apatite present in bone, is a potential bone substitute, but its use is limited to low-load applications because of its poor mechanical strength. Much research on modifying the surface properties of calcium phosphate apatite including HA has been conducted, particularly using plasma techniques. For instance, plas-
ma spraying of apatite onto metallic materials is used to form apatite to enhance active bone formation and bone-conductivity, and an example is plasma sprayed HA coatings on metallic joint prostheses.

Unfortunately, the apatite/titanium interface or apatite itself can be fractured even under relatively low stress because of the low strength and brittleness of the sprayed layer itself.

Although HA has similar chemical composition to that of natural bone, it lacks sufficient strength and durability for use in load-bearing applications. Very often it requires blending with a low modulus polymer to achieve adequate strength. However, the properties of the HA composite are highly dependent on the particle size and morphology of the HA filler. The use of nanoparticles to obtain superior mechanical properties has been proposed. Kumar et al. synthesized ultra-fine HA powder using a plasma technique. The synthesis is initiated using rf plasma spraying onto a wet suspension of HA. It is then axially injected into the rf plasma at various powers (plasma energies), chamber pressure, probe distances, and plasma gas flow rates. The size of the processed powders varies from medium to ultra-fine. In general, the particle size decreases with increasing power.

Decomposition into other phases such as tricalcium phosphate (TCP), tetracalcium phosphate (TTCP) and calcium oxide (CaO) also increases with higher power. The study suggests that the processing parameters associated with the production of ultra-fine powders interact in a complex manner, but can be envisioned as an overall thermal treatment of the particulates. Zheng et al. have also prepared HA/Ti composite coatings by atmospheric plasma spraying to improve the bond strength. Yttrium stabilized zirconia (YSZ) reinforced HA coatings have been shown to enhance the mechanical properties of HA coatings significantly and to reduce the formation of calcium oxide (CaO) in clinical applications. Interface mechanical failure is a potential weakness in prostheses. Fu et al. used spray HA/YSZ composite coatings by plasma techniques to improve the interfacial strength between the HA coating and titanium alloy substrate. Their experimental results show that spheroidized powders melt more effectively than ball milled powders during plasma spraying and give rise to better mechanical properties. An HA/YSZ solid solution forms during deposition and plays an important role in the enhancement of the mechanical properties of the HA/YSZ composite coatings.

In addition to apatite, the inorganic part of natural bone also contains bTCP and several ions such as Na⁺, Mg²⁺, K⁺ and F⁻, and glasses in the P₂O₅-CaO-Na₂O system have been considered to have good potential as biomaterials because of these inorganic constituents. Research on multilayered coatings composed of mixtures of HA and P₂O₅-based bioactive glasses reveals their potential clinical benefits in orthopedic and dental surgery, and pre-immersion of these materials has been reported to further enhance their efficacy in vivo, although the precise biological effects of this treatment are not yet understood. Ferraz et al. have prepared double-layer coatings by plasma spraying and evaluated the effects of pre-immersion on the growth and function of human osteosarcoma cells in vitro. The results show that the number of viable cells on the pre-immersed HA and glass-reinforced HA coatings is the same or higher following incubation compared to the non-immersed materials.

Plasma-sprayed coatings have been applied to orthopedic prosthetic components. However problems cited with the plasma-sprayed coatings include variation in bond strength between the coatings and the metallic substrates, alterations in the HA structure due to the coating process, and poor adhesion between the coating and metallic substrate. Current research on sputtered CaP coatings has shown promise for eliminating some of the problems associated with the plasma-spraying process. It has been generally accepted that sputtered HA and CaP coatings improve bone strength and initial osseointegration rate. Investigations of the sputtering process should include clinical trials, to develop understanding of bone response to coated-implant surfaces with different properties.

2.2.2 Preparation of bioactive inorganic surfaces combining plasma coating and biomolecule immobilization

Although metal and inorganic material surfaces have shown a high degree of bio-inertness, recent investigations have shown that it is also possible to introduce bioactive species on the surface by means of plasma and ion beam processing combined with other technologies.

Ge and Chen et al. synthesized Ti-O films on (100) silicon wafers using an unbalanced magnetron sputtering (UBMS) system. The Ti-O films were then pretreated with 2M sodium hydroxide solution for 3 hours at 50°C to introduce -OH groups onto the Ti-O surface to facilitate subsequent protein immobilization. The films were then washed and air-dried for further laminin immobilization. Laminin immobilization of the Ti-O film surface was achieved by an aminosilane linker. Silanization was first performed by incubating the films in 1% solution of (3-aminopropyl)-triethoxysilane (APTES) in anhydrous alcohol for 48 hours at 60°C. The films were washed in alcohol by a heating regurgitant device. The Ti-O films were then incubated in 40 µg/ml laminin solution for 12 hours at room temperature. The reaction scheme for this procedure is shown in Fig. 14.

Human umbilical vein endothelial cells (HUVEC) cells were seeded on the laminin-immobilized Ti-O film sample surfaces at a certain cell concentration and cultured for 72 h. Subsequently the samples were rinsed, fixed, dehydrated and critical point-dried, and then examined by SEM to observe the adherent state of endothelial cells on different samples, as shown in Fig. 15. This study showed that extracellular matrix (ECM) protein of laminin has been immobilized on a Ti-O film surface, and that laminin immobilized Ti-O films greatly enhanced cell adhesion and growth.

A similar process was performed by immobilizing heparin on titanium oxide films synthesized using a sputter-
tering process\(^{83}\)). Anatase TiO\(_2\) film was treated with phosphoric acid solution to form hydroxyl groups on the sample. APTES (3-Aminopropyltriethoxysilane) then bonded to the film by reaction between ethoxyl of the linking reagent and hydroxyl of the film. As the bifunctional linking reagent, the amino groups of APTES provide the opportunity of further chemically bonding heparin with carboxyl groups. By means of this treatment, the anticoagulation behavior of the surface was significantly improved.

### 3. Conclusion

Plasma and ion beam processing as surface treatment techniques are becoming progressively more common in biomaterials engineering. The important advantage of plasma surface modification is the ability to change the surface properties to become more biocompatible or to have higher durability without altering the bulk attributes, thereby offering a high degree of quality control, yield, reliability and reproducibility that would be difficult using other conventional techniques. By combining plasma and ion beam processing with other techniques such as biochemical processing, it has been possible to prepare biomimetic surfaces of implants to significantly enhance the degree of acceptance in the tissue environment. This direction will develop broadly.

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