Functionalization of biomedical materials using plasma and related technologies

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\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

Plasma techniques are important to biomedical engineering and surface modification. By modifying selective surface characteristics, conventional materials can be designed with superior biological properties while the favorable bulk materials properties can be retained. In this mini-review, recent progress pertaining to surface modification of Mg-based and polymer-based biomaterials by plasma-based techniques such as gas or metal ion implantation, dual metal and gas ion implantation, as well as plasma immersion ion implantation and deposition is described. Plasma-based surface modification is promising in elevating the cell biocompatibility, blood compatibility, and antibacterial properties of Mg-based and polymer-based biomaterials and expected to be extensively applied to biomaterials.

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1. Introduction

Mg-based materials were first introduced to orthopedic and trauma surgeries about one hundred years ago \cite{1}. Compared to stainless steels, titanium, and titanium alloys, magnesium alloys have unique natural biodegradability in the physiological environment and stimulatory effects on new bone formation. Since Mg alloys have an elastic modulus similar to that of human bone, they produce less stress shielding and their natural degradability obviates the need for a second surgical process to remove the implants from patients thereby minimizing trauma and medical costs \cite{2–4}. However, the major obstacle hampering their clinical use is rapid and uncontrolled degradation inside the physiological environment and local gas emission in vivo \cite{5–7}.

Medical polymers are used widely in biomedical implants such as bone substitutes, artificial heart valves, and artificial blood vessels because of their excellent mechanical properties and moderate biocompatibility \cite{8,9}. However, common biopolymers such as polytetrafluoroethylene (PTFE) \cite{10}, polyetheretherketone (PEEK) \cite{11,12}, and poly(butylene succinate) \cite{13} are bio-inert. These materials tend to induce the formation of soft tissues rather than direct bone integration, resulting in fibrous encapsulation of the implant surface. In addition, infection of medical devices can be a life threatening complication and lead to significant morbidity and mortality. Therefore, it is crucial to improve the biocompatibility and antibacterial properties of medical polymers.

In order to obtain optimal biological performance, it is usually necessary to apply surface treatment or coatings to biomaterials. Among the various surface treatment and coating techniques, plasma-based techniques such as plasma ion implantation and plasma immersion ion implantation and deposition (PIII&D) have been proven to be effective in improving the surface properties of materials while the favorable bulk materials attributes like mechanical strength, robustness, and inertness can be preserved \cite{14–17}. By varying the implantation energy, excellent adhesion between the surface modified layer and substrate can be achieved as there is no distinct interface between the implanted layer and substrate. This process is controllable and reproducible and can be tailored to produce different types of surfaces in a desirable way \cite{18}. In this mini-review, recent work conducted on plasma-based surface modification of biomaterials is described. Topics discussed include the cytocompatibility, hemocompatibility, and antimicrobial properties of plasma-treated magnesium alloys and medical polymers as well as corrosion resistance of plasma-treated biodegradable metals.

2. Gaseous plasma ion implantation

Different from conventional beamline ion implantation, plasma immersion ion implantation (PIII) circumvents the line-of-sight restriction and possesses a number of advantages such as simplicity, low cost, efficiency, large area, and batch processing \cite{19}.

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and has been applied to the improvement of surface mechanical properties and biocompatibility of biomedical polymers and metals [20]. The purpose of gaseous PIII into magnesium is to form a barrier layer on the surface to inhibit attack by aggressive ions in the physiological medium and decrease the degradation rate. Hydrogen [21], oxygen [22,23], nitrogen [24], and water [25] have been hitherto plasma implanted into magnesium alloys, as illustrated in Fig. 1 [26]. It has been found that nitrogen PIII can effectively improve the corrosion resistance of magnesium alloys if the implantation conditions are proper [24]. Different implantation parameters may produce adverse effects on the corrosion resistance. During lower energy ion implantation, the original oxide layer is eroded by ion bombardment thus reducing the natural blocking capability and consequently, the sample is prone to corrosion. High dose with high energy ion implantation may degrade the corrosion resistance due to the formation of a large amount of Mg₃N₂ which is sensitive to atmospheric moisture [27]. Hence, it is important to choose the proper implantation conditions in nitrogen PIII. Recently, oxygen PIII [22,23] and water PIII [22,23] have been reported. Although oxygen PIII and water PIII increase the thickness of the surface oxide film, effective protection cannot be provided because the Pilling-Bedworth ratio of magnesium oxide is less than 1 and no significant improvement in the surface corrosion resistance is observed [22]. In contrast, hydrogen ion implantation appears to be more effective due to the formation of corrosion resistant MgH₂ [21]. Although a variety of gas plasma treatment has been applied to magnesium alloys, the related biological properties and pertinent enhancement mechanisms are still not well known.

Gaseous PIII can be conducted on polymers to improve the cytocompatibility, blood compatibility, and/or antibacterial properties. Precise control of the processing parameters can modulate the surface characteristics of polymers such as surface topography, chemical composition, and surface hydrophilicity thereby enabling the polymer to meet the requirements for particular applications.

Up to now, nitrogen [28–31], oxygen [10,32], hydrogen [33], argon [30,34,35], and helium [36,37] have been implanted into polymers to enhance the biological properties. Nitrogen PIII produces nitrogen-containing functional groups on the polymer surface. For example, the modified polyethylene (PE) surface has antibacterial properties and the ability to enhance osteoblast differentiation [28]. The enhanced biocompatibility may be related to the new

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**Fig. 1.** Schematic illustrating the process of plasma surface modification. A gas is introduced into the evacuated chamber and then ionized by a plasma source. The charged species then impact the substrate to modify the surface in the immersion configuration [26].

**Fig. 2.** (a) Schematic of a yeast cell wall attached to a PIII-treated surface. The proteins from the cell wall are covalently bound to the modified surface (covalent bonds are indicated by solid line segments). (b) Schematic of a rehydrated yeast cell wall attached to an untreated surface. The lipids and proteins are physisorbed on the hydrophobic surface [29].
covalent bonds which effectively increase the quantity of attached functional proteins on the PE. Similarly, the collagen on nitrogen PIII PTFE possesses better adhesive activity than the untreated surface [38]. The mechanism of protein attachment is based on the creation of long-living free radicals and/or reactive groups created by the reactions between the radicals and ambient oxygen and/or water vapor to create functional binding groups, e.g. carbonyl, that are able to bind covalently to proteins [30]. Tran et al. [29] have analyzed the different cell attachment on plasma-treated and untreated PTFE surfaces. Rapid attachment on the PIII surface stems from covalent bonds of cell wall proteins and radicals on the treated surface. In contrast, on the untreated surface, only physically adsorbed molecules are found from the residue and lipids are more concentrated than proteins. The presence of lipids in the residue is a consequence of damage to the plasma membrane during rehydration, as shown in Fig. 2. The research conducted so far suggests that nitrogen PIII may be used to manipulate the biological response on PTFE and PE and the improved materials performance bodes well for tissue engineering applications. In addition to the biocompatibility enhancement, the anticoagulation and anticalcification behavior on polymers such as poly(methyl methacrylate) (PMMA) surface can be enhanced by nitrogen ion implantation [31]. The loss of some original chemical bonds on the surface and formation of new nitrogen-containing groups are considered to be responsible for the enhanced blood compatibility on PMMA.

The hydrophilicity of poly(butylene succinate) can be significantly improved by oxygen PIII and the modified samples exhibit enhanced compatibility to osteoblasts [13]. A long pulse, high frequency oxygen PIII process conducted in the presence of a shielded grid to reduce sample charge can effectively up-regulate the OCN expression of osteoblasts on PTFE. The effects are better than those obtained by conventional oxygen PIII from the perspective of using PTFE in bone or cartilage replacements [10]. Argon ion implantation has been studied extensively as well. XPS (Table 1) and FT-IR (Fig. 3) reveal larger amounts of functional groups such as OH, C=O,

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<th>Ion Fluence (ions/cm²)</th>
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Table 1. Surface composition (atomic %) of the pristine PC and the Ar⁺ implanted PCs determined by XPS [35].

Fig. 3. FT-IR spectra: (a) Pristine PC, (b) Ar⁺ implanted PC at fluence of 1 × 10¹⁴, (c) Ar⁺ implanted PC at fluence of 1 × 10¹⁵, and (d) Ar⁺ implanted PC at fluence of 1 × 10¹⁶ ions/cm² [35].

Fig. 4. Fluorescent images of MC3T3-E1 pre-osteoblasts after culturing for 5 h: (a) unimplanted Mg–Ca, (b) Zr-implanted Mg–Ca, (c) Zr–O-implanted Mg–Ca, (d) unimplanted Mg–Sr, (e) Zr implanted Mg–Sr, and (f) Zr–O-implanted Mg–Sr alloy.
and C–O on the argon ion implanted polycarbonate (PC) surface and improved surface hydrophilicity [35]. The in vitro study shows that the quantity of attached functional protein on the plasma treated surface increases and cells preferentially adhere to the ion implanted regions on the polymer [34,35]. Moreover, argon PIII has an enhancement effect on the attachment of human skin fibroblasts F1544 to chitosan membranes also suggesting enhanced cytocompatibility after ion implantation [39] and good antibacterial properties are observed from the polymer surface after oxygen and argon PIII [40].

Besides common nitrogen, oxygen and argon ion implantation, the biological property of polymer surface can be enhanced by helium or hydrogen ion implantation. Kurotobi et al. [36] have observed that helium ion implantation can effectively improve the antithrombogenicity of collagen-coated polystyrene tubes. Hwang et al. [33] have reported that the cell-resistant Pluronic surface can be converted into a cell-adhesive one by hydrogen ion implantation and his process offers the micro-patterning capability of two different cell types on the polymer substrate.

3. Metal ion implantation

Metal ion implantation is an effective technique to enhance coating adhesion and surface properties by taking advantage of both ion mixing and formation of graded near-surface structures. Several metallic ions have been implanted into magnesium and its alloys to modify the surface composition and improve corrosion resistance for biomedical applications, for instance, Zr [41], Ti [42,43], Zn [44,45], Si [46], Ta [47], Cr [48], and Al [49]. After Zr, Ti, Si and Al ion implantation, the corrosion resistance of Mg and Mg alloys is enhanced appreciably. Ion implantation produces a thick modified layer and because of the retardation effects of metal atoms, magnesium diffusion from the substrate to the surface is restricted and the magnesium content in the near surface is dramatically reduced. In addition, a corrosion-resistant metal oxide is formed during the process. Reduction in the amount of reactive magnesium and formation of a corrosion-resistant metal oxide enhance the corrosion resistance synergistically [50]. In the physiological environment, the proper degradation rate of magnesium not only creates a relatively stable interface for cell adhesion and growth but also retards the release of corrosion products to reduce the cytotoxicity [51].

Selection of the proper implanted elements is critical. The implanted elements should be non-toxic and after ion implantation, the corrosion resistance of the Mg alloy should be enhanced significantly. Al [49] ion implantation offers good corrosion resistance because of the strong affinity of aluminum with oxygen resulting in the formation of a stable passive aluminum oxide layer on the surface. Nevertheless, although it is still controversial, aluminum is suspected to have adverse effects on the nervous systems and may cause Alzheimer’s disease [52] and its use must be considered carefully. Zn [44,45] or Cr [48] ion implantation accelerates corrosion on Mg and Mg alloys in simulated body fluids and are not suitable if slower degradation is the objective.

As described in the previous section, medical polymers are used extensively in biomedical devices because of the good mechanical and biological properties. However, bacterial infection of medical devices can lead to life threatening complications and subsequently significant morbidity and mortality. Therefore, it is important to improve the antibacterial properties on polymers. In this respect, metal ion implantation is a good technique. It has been found that the surface antibacterial properties of polyethylene are improved by Ag PIII [53,54] and Cu PIII [9]. Ag PIII produces nearly 100% bactericidal effects on PE whereas Cu PIII yields a slightly smaller value of about 95% [55].

Fig. 5. Cell proliferation evaluated by the fold change of the incorporation of BrdU on the unimplanted, Zr-implanted and Zr–O–implanted binary magnesium alloys after culturing for 1 and 3 days. The data are normalized to the unimplanted control: (a) Mg-Ca alloy and (b) Mg–Sr alloy. Statistically significant differences at *p < 0.05 vs. control.

4. Dual metal and gas plasma ion implantation

In metal ion implantation, improvement in the corrosion resistance is often related to the formation of a corrosion-resistant metal oxide and dramatically reduced magnesium content in the near surface [50]. However, in many cases, it is still necessary to modify the sample surface further after metal ion implantation to enable optimal formation of the metal oxide. In this respect, oxygen co-implantation can yield the optimal effects. Under high-energy bombardment, oxygen ions penetrate the surface and react with the implanted metals such as Zr, Ti, Al, and Cr as well as magnesium simultaneously. The process produces a protective oxide film composed of ZrO2, TiO2, Al2O3, or CrO3 [41,42,56–58] to enhance the surface corrosion resistance. The structure of the modified layer after dual metal and oxygen ion implantation is also more ideal. For example, the mismatch in the mechanical properties between the metal oxide and Mg can be reduced because the implanted metal layer serves as a transition layer between the metal oxide layer and magnesium substrate [56].
Dual zirconium and oxygen ion implantation improves both cell adhesion (Fig. 4) and proliferation (Fig. 5) on the binary Mg–Ca (or Mg–Sr) alloys [41]. The improved biological response is believed to be related to the better corrosion resistance, since the cytotoxicity of magnesium alloy is normally attributed to the large degradation rate and subsequent interfacial reaction between the unimplanted Mg–Ca (or Mg–Sr) and corrosive medium to adversely affecting cell adhesion and growth. After dual zirconium and oxygen ion implantation, the ZrO$_2$-containing surface layer serves as a barrier to retard corrosion. The reduced degradation rate on magnesium not only creates a relatively stable interface for cell adhesion and growth, but also mitigates the release of corrosion products that can lead to cytotoxicity. In addition, dual zirconium and oxygen ion implantation enhances the antimicrobial properties of Mg–Ca and Mg–Sr alloys (Fig. 6). In the physiological environment, the positively charged metals (Zr$^{2+}$ and Mg$^{2+}$) interact with the negatively charged microbial cell membranes. This may alter the cell permeability or disruption of the membrane integrity, ultimately resulting in the leakage of proteinaceous and other intracellular constituents [59]. Micro-galvanic effects can be triggered by metal ion implantation in the physiological environment because zirconium and magnesium have different standard electrode potentials. The proton-depleted regions produced by the micro-galvanic coupling reactions can inactive ATP synthesis, ion transport, and metabolite sequestration, ultimately leading to bacteria death [60].

The superior cytocompatibility and antibacterial properties suggest that biodegradable magnesium alloys with a plasma-modified surface have good potential as orthopedic implants.

Even though Ag or Cu ion implantation can inhibit bacteria adhesion or kill bacteria, these metals do not bond with the polymeric matrix or form radicals. Hence, Ag or Cu ion implantation only endows PE with temporary antibacterial ability and the antibacterial effects diminish rapidly with time because the metals leach rapidly from the implanted polymer [61]. It has been observed that NH$_3$, O$_2$, or N$_2$ co-implantation in concert with Cu ion implantation into polyethylene can regulate the copper release rate from the substrate and enhance the long term antibacterial effects [62,63].

Chemical state analyses demonstrate that plasma implantation produces polar functional groups of C–O, C=O, C=N, C≡N, and C≡N which are believed to play important roles in regulating Cu out-diffusion producing the best long term antibacterial properties. Among the three gas species, NH$_3$ PIII produced C=NH$_2$ groups which do not offer free bonds to prevent the metal from leaching, thus showing the least in regulating the Cu leaching rate whereas the N$_2$ plasma produces the best results due to more effective C≡N and C≡N bonds in the Cu/N$_2$ PIII PE. Similar antibacterial effects have been observed from Ag/N$_2$ PIII polymers [53]. In general, the choice of the gaseous element in PIII after Ag or Cu ion implantation is crucial to the antibacterial characteristics of the polymers.

5. Plasma immersion ion implantation and deposition (PIII&D)

Plasma immersion ion implantation and deposition (PIII&D) is an advanced surface modification technique involving energetic ion implantation, low-energy plasma deposition, and formation of an atomically intermixed layer between the substrate and coating [19,64]. During PIII&D, single or multiple plasmas are used. When a cathodic arc metal plasma source is used to introduce the metal ions, the process can be adjusted to achieve pure implantation, pure deposition, or in between by adjusting the target voltage and synchronizing the high voltage pulses [65]. It has been found that the corrosion resistance of AZ91 magnesium alloy is improved by titanium, aluminum and zirconium PIII&D [66]. After the process, a tri-layered microstructure with an outer layer composed of mainly oxide with a small amount of MgO and Mg(OH)$_2$, an intermediate layer containing metal oxide and metallic implanted particles, and a bottom layer rich in metallic elements is formed. Electrochemical corrosion tests indicate that Al PIII&D offers better corrosion protection than Ti PIII&D and Zr PIII&D. Considering the superior corrosion protection offered by Al$_2$O$_3$, dual aluminum and oxygen PIII&D has been conducted to suppress rapid corrosion, leaching of magnesium ions, as well as hydrogen gas release from biodegradable alloys in the physiological environment [67]. After PIII&D, a
graded modified layer is formed between the substrate and coating. The plasma-treated implant degrades slowly in vivo and the small amount of released magnesium ions can effectively stimulate new bone formation after surgery (Fig. 7). These promising results demonstrate the potential of plasma-treated magnesium implants in treating diseases associated with bone loss.

PIII&D has also been utilized to prepare diamond-like carbon (DLC) films. DLC materials possess not only excellent tribological and mechanical properties but also good cytocompatibility and hemocompatibility [68–73]. Osteoblast attachment, proliferation, and differentiation have been observed to be superior on DLC coated polyetheretherketone (PEEK). The DLC/PEEK system is almost isoelastic to cortical bone thus bonding well for orthopedic applications [11]. Recently, Wu et al. [74] have found that DLC films with a thickness of about 200 nm can significantly improve the corrosion resistance of Mg–Nd–Zn–Zr alloy in a 0.9 wt% NaCl solution suggesting wide applications to biomedical engineering. DLC doped with a proper amount of nitrogen, phosphorus, or fluorine can improve the surface blood compatibility and decrease the cytotoxicity [75–77]. These and other results suggest that PIII&D is an effective surface modification method for the enhancement of biological properties of Mg-based and polymer-based biomaterials.

6. Conclusion

Plasma surface modification is a versatile technique in biomaterials engineering. One of the advantages is that the surface biological properties can be selectively enhanced without changing the bulk properties of the biomaterials. The recent progress in gas or metal ion implantation, dual metal and gas ion implantation as well as plasma immersion ion implantation and deposition of Mg-based and polymer-based biomaterials is reviewed. The results show that plasma-based surface treatment conducted under the optimal conditions can produce superior surface cytocompatibility, hemocompatibility, and antibacterial properties.

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