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Graded metal carbon protein binding films prepared by hybrid cathodic arc — Glow discharge plasma assisted chemical vapor deposition

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ABSTRACT

Graded composite layers containing metal and plasma polymer components were deposited using a cathodic arc in conjunction with plasma immersion ion implantation. Using a bias potential throughout, pure metal was deposited initially using the cathodic arc alone and then acetylene was added to the process to increase the fraction of the plasma polymerized carbon film. To test adhesion, the substrate and film were strongly deformed by folding the substrate inward and outward with a small radius of curvature. Strong adhesion between the metal surface and the deposited layers was achieved by the use of the graded layers as inferred from the SEM observations of the deformation region. Strong adhesion of biologically active protein molecules to the surface of the graded layer was confirmed by detergent washing and colorimetric enzyme activity assays. These characteristics suggest that the coatings may be suitable for cardiovascular stent applications.

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1. Introduction

Immobilizing proteins on surfaces by means of covalent binding while retaining their function over a long period of time is increasingly in demand for many biomedical and biochemical applications such as biosensors and medical implants [1–6]. For implantable medical device applications, immobilizing proteins by utilizing physical interactions renders the proteins vulnerable to detachment and undesirable conformation changes which affect their bioactivity can lead to adverse responses such as inflammation, clotting, activation of cellular immune response, excessive fibrosis, or rejection. During heart transplantation using cardiopulmonary bypass heart-lung machines, patients exhibit an inflammatory response which is characterized by increased expression of at least ten leukocyte cluster-of-differentiation (CD) antigens [7]. Coronary artery disease is primarily caused by the inflammation of coronary vessel walls [8] and a study on coronary stents provided strong evidence that increased inflammation can arise from the use of bare stents [9]. To minimize these inflammatory responses, attempts have been made to cover surfaces which are exposed to blood with protein molecules such as albumin derived from human blood plasma. However, these attempts have frequently been unsuccessful [10]. The lack of success may be attributed to difficulties in achieving strong protein binding or causing denaturation (conformational change) of the molecule. Maintaining the native conformation of an immobilized biomolecule is critical in situations where the active site involves amino acid residues that are proximate due to the molecule's energetically favored folded state in aqueous biological environments. In such cases, varying of conformation state of a molecule can adversely affect the biorecognition process [11].

Plasma modification technologies have been found useful to improve surface properties [11,12] such as imparting a covalent protein binding capability [1,13–16], corrosion resistance [17,18], biocompatibility and antimicrobial [19] properties of biomaterials.

Surface energy is an important characteristic that determines the strength of physical adsorption and the degree of retention of native conformation of protein molecules. Highly hydrophilic surfaces such as polyethylene glycol do not bind proteins [20] whereas hydrophobic surfaces adsorb proteins relatively strongly but typically induce changes in their conformation. Hydrophilic surfaces, while desirable to preserve protein function [21], have low protein binding affinity [22]. Bilek et al. [1] demonstrated a plasma ion-assisted processes which can enable covalent binding to hydrophilic surfaces. The plasma modification was shown to generate mobile unpaired electrons associated with free radicals and these were shown to be the mechanism for the formation of covalent bonds with biomolecules. Yin et al. [2] demonstrated one such carbon plasma deposition process on a stainless steel (SS) substrate which provided a robust interface for covalent immobilization

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of tropoelastin while Bax et al. [23,24] demonstrated a pure implantation process to covalently immobilize a range of extra-cellular-matrix proteins to PTFE. The immobilized tropoelastin protein molecules enhanced the attachment of endothelial cells and improved their proliferation.

Amorphous carbon films are well-known as being biocompatible [25–29] and hence it is an attractive candidate for covalent immobilization of protein molecules [2,15,30,31]. Liu et al. [25] studied that amorphous carbon film stimulates lower inflammatory reaction and higher osteoblast viability. Wei et al. [26] studied that amorphous carbon film promotes the human endothelial ECV304 cell growth. Ma et al. [27] studied that amorphous carbon film suppressed the viable monocyte/macrophages attachment. Kwok et al. [28] studied that silver doped amorphous carbon film improves the hemocompatibility and antibacterial properties. Dowling et al. [32] reported that after 4 weeks, amorphous carbon film implant had no macroscopical adverse effects on the cortical bone or muscular tissue of sheep under in vivo conditions. Zolynski et al. [33] reported that after 28 weeks, amorphous carbon film implant enables the healing of bone fracture under in vivo conditions in the human body. Waterhouse et al. [15] reported that amorphous carbon film covalently immobilizes protein molecules and reduces thrombogenicity. Yin et al. [2] reported that amorphous carbon film covalently immobilizes protein molecules. However, in the case of metallic materials such as stainless steels, adhesion between carbon-based plasma polymers and the metal is a major problem because of the different thermal expansion coefficients between the two materials. Yin et al. [30] deposited a carbon-based plasma polymer film using a graded interface between the metal and carbon containing polymer which displayed robust covalent immobilization of protein as well as exceptionally strong adhesion (between 18 and 26 MPa) to the underlying metal substrate.

Strong adhesion is desirable for biomedical implants such as cardiovascular stents that undergo major deformations during surgical insertion. Graded layers can solve the adhesion problem between metallic materials and a carbon layer provided that the metal layer that is initially deposited as part of the graded layer adheres well to the metal substrate. Cathodic arc deposition, a type of physical vapor deposition, is suitable for achieving well adhered metal layers because of the high ion content of the depositing materials, thus enabling the metal ions to be implanted into the metal surface in a process known as plasma immersion ion implantation and deposition (PIII&D). This type of deposition produces strong adhesion of the coating to the underlying metallic substrate [34–36]. A well adhered gradient metal-carbon layer deposited using a cathodic arc process and enabling covalent binding of biomolecules has not previously been reported. The aim of this work is to demonstrate that cathodic arc deposition combined with plasma immersion ion implantation (PIII), and acetylene plasma assisted chemical vapor deposition (PACVD) with PIII provides an adherent graded composition coating with covalent binding capability for the immobilization of biologically active proteins.

2. Methods and materials

2.1. Plasma deposition processes

Films containing Ti and C were deposited in a cathodic arc deposition chamber (Fig. 1) fitted with a titanium cathode of 99.998% purity. A curved magnetic solenoid filter of the type described by Aksenov et al. [37] was used to filter neutral atoms and macro-particles from the plasma stream. 10 A current was applied to the field coils of the magnetic filter, consisting of two layers of wire with n turns per meter. More details about the chamber have been reported earlier [38–40]. Briefly, two power sources were used: (1) a low voltage and high current (set to 60 A) source to power a cathodic arc which generated the metal vapor plasma, and (2) a pulsed voltage source, set at −2 kV, 20 μs pulse width, and 3 kHz pulse repetition frequency, for biasing the

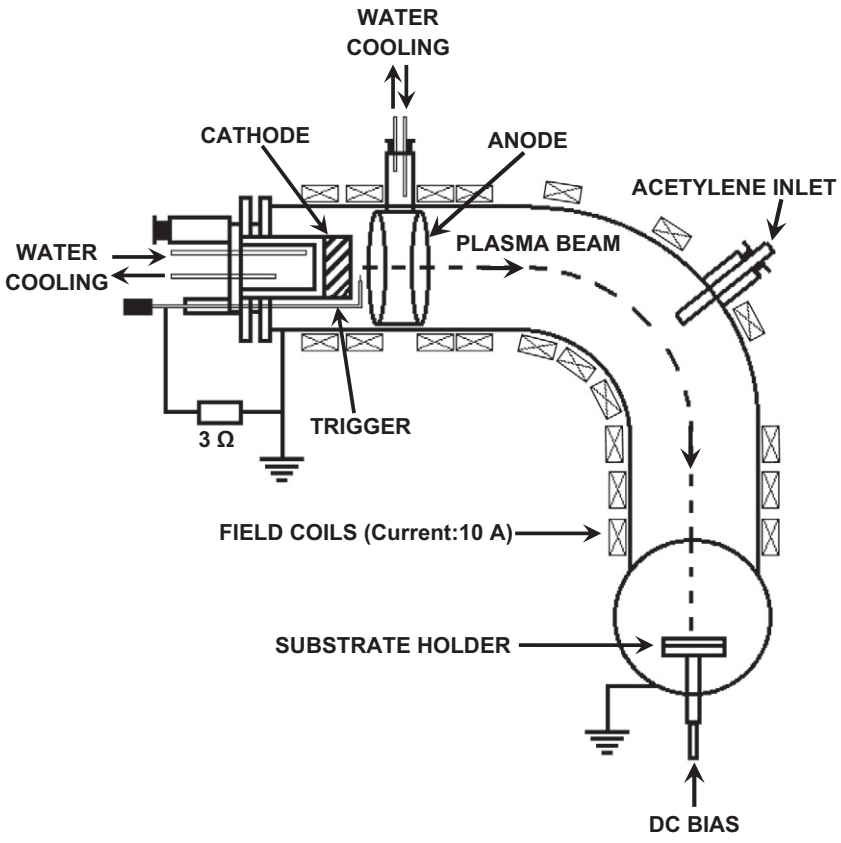

Fig. 1. Schematic illustration of cathodic arc chamber.

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