

Amine Rich Plasma Polymerization using Inverter Plasmas for Orthopaedic Application

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Abstract: In the present study, amine-rich plasma polymerization on hydroxyapatite (HA) artificial bone surfaces was performed prior to bone grafting to support bone cell growth and bone healing with the use of pulsed plasmas called inverter plasmas. Plasmas used for polymerization were characterized by optical emission spectroscopy (OES) and the surface chemical states of deposited polymers were analysed by X-ray photoelectron spectroscopy (XPS). It has been found *in vitro* and *in vivo* that amine-coated HA artificial bones have enhanced osteogenic ability.

Keywords: plasma deposition, artificial bone, amino groups, osteogenesis

1. Introduction

Recently plasma surface modification has been widely used to improve surface functionalities of scaffolds made of, e.g., polymers [1][2]; one of such applications is the surface modification of hydroxyapatite (HA) artificial bones, which support cells and tissues in osteogenesis. In a previous study, hydrophilicity on HA was shown to improve by the treatment of dielectric barrier discharge (DBD) plasmas with a mixture of O₂ and He gases [3]. In this work, we have explored it further by investigating effects of amino groups formed on HA artificial bone surfaces using plasma polymerization. We have also studied depth profiles of N-containing polymers inside porous HA artificial bones by cross section analysis and the effect of standard sterilization processes of artificial bones on amino group functionalities formed on plasma-polymerized surfaces.

2. Preparation of amine films

Hydroxyapatite (HA) artificial bones were supplied by Aimeedic MMT, which have molecular formula Ca₁₀(PO₄)₆(OH)₂, purity of 99.9%, porosity 72%-78%, and average pore diameter of 150 μm. Each sample has a circular disk shape with a diameter of 5mm and a thickness of 2 mm. Plasma polymers were deposited on the HA artificial bones by using inverter plasmas. Inverter plasmas were produced by a pulsed high voltage power supply [4] with a 5 kHz alternating electric field between the parallel electrodes. The base pressure of the plasma system was maintained below 5×10⁻³ Pa via a turbo-molecular pump. In the plasma polymerization process, the HA samples were cleaned for 10 min by an Ar plasma at a pressure of 70 Pa and then exposed to a plasma based on a CH₄, He, and N₂ mixed gas at a pressure of 70 Pa, with a voltage

(V_{pp}) of 2.4 kV and a pulse width of 1 μs. A schematic diagram is given in Fig. 1.

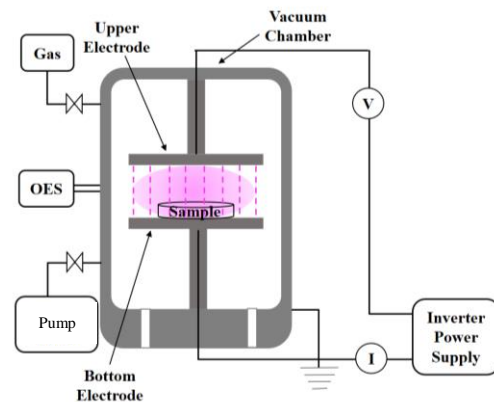


Fig. 1. A schematic diagram of the inverter plasma system

3. Plasma diagnostics of depositing plasma

We performed optical emission spectroscopy (OES) during plasma deposition. In Fig. 2, a typical OES measurement is shown for wavelength between 300 nm and 700 nm. The N₂⁺ first negative system ($B^2 \Sigma_u^+ - X^2 \Sigma_g^+$) was found to be the most significant signal from emitting plasma because of the intense emission of N₂⁺ (0,0) band head at 391.4 nm. Other band heads (0,1) and (0,2) that could be clearly assigned to N₂⁺ were found at 427.8 nm and 470.8 nm, respectively, whereas another possible signal at 358.2 nm overlapped with the second positive N₂ system ($C^3 \Pi_u \rightarrow B^3 \Pi_g$), the transition (0,1) at 357.7 nm, that was likely to account for the peak intensity more predominantly [6,7]. Other significant contributions from the second positive N₂ system were found at 315.9 nm, 337.1 nm, 353.7 nm and others as shown in Fig. 2.

The OH emission between 306 nm and 330 nm, seen as an impurity in the gas mixture, overlapped with N₂ spectrum at 315.9 nm [6]. The high intensity of N₂⁺ is somewhat surprising because Clay *et al.* [7] observed an enhancement of N₂ emission relative to N₂⁺ after addition of CH₄ to N₂ in RF discharges. The peak at 399.5 nm was assigned to N⁺. This line is the most intense of N⁺ emission lines [7] but yet its presence in N₂-containing discharges has been seldom reported.

The CN violet system ($B^2\Sigma - X^2\Sigma$), having the most intense band head at 388.3 nm, could be assigned to an extra peak appearing in the strong N₂⁺ emission. It is often observed in carbon and nitrogen containing plasmas [7,8]. In CH₄/N₂ plasmas it can be a product of dissociative reaction of excited CH₄ and N₂ or a product of the dissociation of intermediate stable compounds containing CN bonding [8]. However, we should keep in mind that N₂⁺ (1,1) appears closely to it at 388.4 nm. Other dissociation product originating from CH₄ was CH whose emission band head was detected at 431.3 nm at very low intensity. The hydrogen line H_α was visible at 656.3 nm [7] but unlike in [6] H_β at 486.1 nm is very weak and hardly detected. We observed also low intensity He lines at 501.6 nm and 667.8 nm.

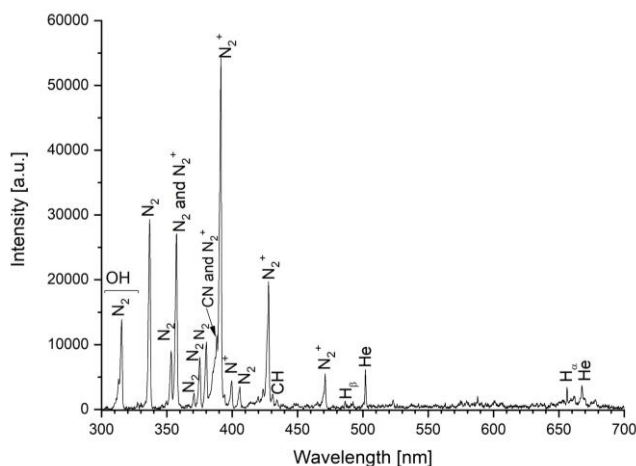


Fig. 2. Optical emission spectroscopy (OES) spectrum obtained from Inverter Plasma

3. Analysis of deposited films

a. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) for chemical characterization on HA surfaces was carried out using XPS Kratos analytical axis supra, escalab 250Xi (SHIMADZU), with a resolution of 0.1-0.2 eV, a pass energy of 20 eV, and Mg-K α 1253.6 eV as the X-ray source. We investigated N-containing functional groups on HA surfaces after plasma

polymerization. The result of XPS analysis has confirmed that the chemical composition on a HA surface prior to plasma deposition is consistent with typical HA stoichiometry with low carbon concentration as impurities on the top surface. After plasma polymerization, the chemical composition in the film deposited on the HA surface was found to be carbon 76.7%, nitrogen 16.5% and oxygen 6.7%. We also observed a similar chemical composition of a plasma polymerized film on a silicon sample, i.e. carbon 79.2%, nitrogen 14.1% and oxygen 6.7%. The atomic concentrations of C and N increased significantly after the plasma treatment, which indicates that N-containing functional groups or amino groups were formed on the artificial bone surface.

b. Infrared spectroscopy

We also analyzed the chemical structures of the deposited films using Fourier-transform infrared (FTIR) Bruker Vertex 80v spectrophotometer with a range of 380-4000 cm⁻¹, resolution 4 cm⁻¹ and 150 scans [6]. The FTIR results have confirmed the formation of amino groups in the film deposited on a Si substrate by inverter plasmas. The relative transmittance was calculated from the transmittance of the film deposited on a Si substrate divided by the transmittance of a bare Si substrate. The results identified spectra of C=C, C=N, N-H bonds at 1575-1636 cm⁻¹, Si-H, C=C, C \equiv N bonds at 2080 cm⁻¹, sp³ CH at 2936 cm⁻¹, and N-H or hydroxyl O-H groups at 3371 cm⁻¹ [2][6][9][10].

c. Ellipsometry

The primary aim of ellipsometric measurement was to get information about the film thickness formed on a crystalline Si (c-Si) substrate. The data were acquired using a Woollam V-VASE ellipsometer in the spectral range from 0.75 to 6.5 eV (wavelength range from 190 to 1650 nm) at four angles of incidence from 60 to 75 deg. The data were fitted by the model described below. The agreement of the fit to the measured data is shown in the bottom part of Fig. 3.

The structural model of the sample consisted of two layers on c-Si substrate (Fig. 4). The lower layer represents a substrate amorphized by ion bombardment and the upper layer represents the deposited film. Fixed tabulated optical constants were used for both crystalline and amorphous Si materials [11]. Optical constants of the deposited film were modelled using a parametrised joint density of states (PJDOS) model consisting of one interband contribution modified by two broad Gaussian terms [12]. The fitting resulted in the film thickness of 44.16 ± 0.11 nm whereas the thickness of the transition amorphous Si layer was 3.54 ± 0.15 nm. However, the optical constants of the film, *layer n* and *k* shown in Fig. 3, differed from typical values of polymer films. They rather represent parameters of some

effective material that represented, in our simplified model, more complicated structures of the coating.

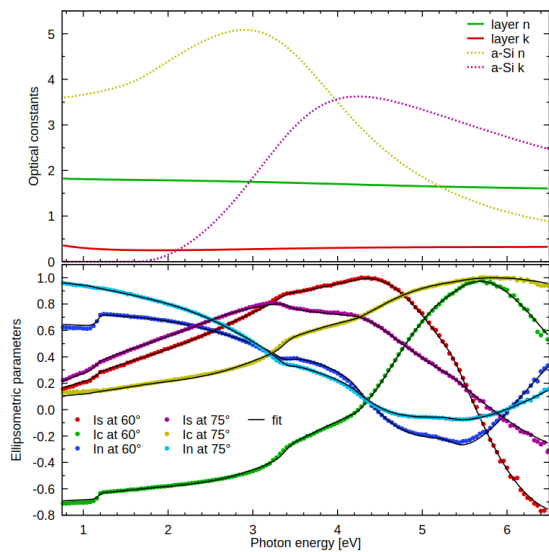


Fig. 3. Ellipsometric parameters and optical constants for the deposited film and amorphized Si layer.



Fig. 4. Illustration of the film thickness on a silicon substrate after plasma deposition

4. Summary

Plasma deposition using inverter plasmas was conducted to form plasma polymerized films with amino groups on surfaces of porous HA artificial bones. It has been found from a derivatization method that a sufficient amount of amine is formed on and inside the deposited polymer films. The formation of amino groups on a porous HA artificial bone improved the hydrophilicity of its surfaces, including the inner pore surfaces of its microscopic pores. Coating of the inner pore surfaces is expected to increase blood flows into the inter-connected pores of the artificial bone *in vivo*. We have also studied depth profiles of N-containing polymers inside porous HA artificial bones by cross section analysis, which have confirmed that chemically reactive species penetrate into microscopic pores and form plasma-polymerized films on their inner wall surfaces during the process, making the inner walls of micro pores more hydrophilic. The osteogenic effects may be compromised by the sterilization processes required at the final stage of artificial bone manufacturing process and we have indeed observed a loss of some amino groups by such processes under certain conditions. If a sufficient amount of amine is

preserved in the final sterilization process, we have confirmed that surface amine coating improves the osteogenic ability of a porous HA artificial bone *in vitro* and *in vivo*. Data that support the claims above but are not shown in this manuscript will be given in the presentation.

Acknowledgements

One of authors, Anjar Anggraini Harumningtyas received a scholarship for M. Eng from Research and Innovation in Science and Technology Project (RISET-PRO), Ministry of Research, Technology, and Higher Education of Indonesia. This research was partially supported by the Matching Planner Program from Japan Science and Technology Agency (JST), the Regional Industry-Academia Value Program from JST, Grant-in-Aid for Scientific Research (S) from Japan Society for the Promotion of Science (JSPS KAKENHI Grant Number 15H05736), and the International Joint Research Promotion Program from Osaka University.

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