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Reprint of "Biological characteristics of human fetal skin fibroblasts and MG-63 human osteosarcoma cells on tantalum-doped carbon films"☆

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ABSTRACT

Tantalum is considered a highly biocompatible metal with high corrosion resistance. Metal-doped amorphous carbon films are gaining interest as an effective surface modifier for medical devices. In this study, tantalum (Ta), TaC/amorphous carbon (a-C), and Ta-containing amorphous hydrogenated carbon films (Ta-C:H) were synthesized using a twin-gun magnetron sputtering system to improve biological properties and explore potential applications for surgical implants or devices. Reactive C_2H_2, activated by the tantalum plasma in the magnetron sputtering process, was used to deposit the Ta-C:H coatings. The deposited TaC/a-C coatings, in which TaC was embedded in the amorphous carbon matrix as a nanocomposite film, exhibited favorable cell viability of the human osteosarcoma cell line (MG-63), similar to uncoated titanium. Among the examined materials, Ta-C:H coatings exhibited the highest biocompatibility for WS1 human fetal skin fibroblast cells derived from soft tissues and demonstrated the highest biological performance.

1. Introduction

Amorphous carbon (a-C) and amorphous hydrogenated carbon (a-C:H) thin films, because of their excellent mechanical and biocompatible properties, have become highly promising materials for surface coating in biomedical applications, especially for vascular stent coatings, hip prostheses, orthopedic implant alloys, and artificial heart diaphragms [1–4]. Amorphous carbon and a-C:H films thin films possess high hardness values, a low coefficient of friction values, chemical inertness, and compatibility with various cells. Many in vitro studies have demonstrated that an inflammatory response or cytotoxicity is barely detectable in cells exposed to a-C and a-C:H thin films, such as glial cells, fibroblasts, and osteoblasts [5–9]. No cellular damage or irregular morphology has been observed. A recent study showed that an a-C coating on artificial hip joints considerably reduced wear debris [2]. Several in vitro studies have also demonstrated that a-C coatings improved the adhesion and growth of osteoblasts, reduced platelet attachment, and inhibited activation [5,10]. Therefore, a-C and a-C:H thin films are considered promising materials for use as surface coatings for various biomedical devices and implants.

The biological and physiological behaviors of implants are strongly and critically influenced by the mechanochemical properties of the implant surfaces. The chemical situations of the implant surfaces and bodily environments further affect cell behavior, including the interaction and adsorption of various proteins, cell migration, adhesion, and differentiation. The release of ions may also cause cytotoxicity in cells. Because of their well-known biocompatible and hemocompatible features, a-C and a-C:H have been widely applied (individually or alloyed with other elements) to several cardiovascular and orthopedic devices [2,11,12]. Amorphous carbon and a-C:H coatings can be alloyed with particular elements, such as Si, F, N, O, W, V, Co, Mo, and Ti, and numerous other combinations. Therefore, a-C and a-C:H coatings can be observed in the application of two main fields: blood-contacting and load-bearing joints. The carbon-based surface chemistry of a-C and a-C:H coatings seems to be beneficial in suppressing metal ion release and thrombus formation in vascular (in blood vessels) applications in stents, heart valves, and blood pumps. However, previous studies have revealed that a-C-coated articulating implants (hip joints and toe joints) fail because of coating delamination after 4 to 10 years in vivo [13,14]. To optimize the biological and chemical properties of a-C used in medical applications, the surface chemistry can be tuned by doping or alloying a-C and a-C:H with other elements. Tantalum (Ta), which possesses high biocompatibility and

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unique mechanochemical properties, may be an alternative element to be doped with a-C in medical applications.

Tantalum (Ta) metal is also well known as an excellent biomedical material that is used to manufacture stents for endovascular surgery and orthopedic applications because of its high fracture toughness, corrosion resistance, and biocompatibility [15–19]. An extremely thin film of tantalum oxide that naturally forms on the surface of Ta metals may provide living cells with added affinity and biocompatible environments [20,21]. Ta-based implants demonstrate high biocompatibility in both soft and hard tissues in in vivo and in vitro tests. The blood compatibility of Ta has been proven, and the Ta film is currently used in artificial heart valves. Shtansky et al. demonstrated that osteoblasts yield a high proliferation rate on Ta-doped nanostructured films and exhibited alkaline phosphatase activity [22]. Previous studies [23–25] have demonstrated that Ta metal is beneficial for osteogenesis in animal implantation tests and suitable for cell adhesion, proliferation, and differentiation in in vitro studies [26–28]. Furthermore, Ta has been used to fabricate stents and artificial heart valves for cardiac and vascular devices because of its high corrosion resistance and radio-opacity property [29,30]. The biocompatibility and hemocompatibility of Ta have been identified and applied to various biomedical materials and devices.

To investigate and compare the potential biocompatibility of Ta-doped carbon films in both hard and soft tissues, in the current study, Ta, TaC/a-C, and Ta-C:H coatings were synthesized using a pulsed unbalanced magnetron sputtering process. The Ta-C:H coating was deposited by introducing reactive C_2H_2, which was activated by the tantalum plasma during the magnetron sputtering process. The biocompatibility test was performed using an MTT assay to evaluate the cell viability of the human osteosarcoma cell line (MG-63, derived from the hard tissue) and human fetal skin fibroblasts (WS1, derived from the soft tissue).

2. Experimental details

2.1. Sample preparation and characterization

Tantalum, composite TaC/a-C and Ta-C:H coatings were deposited on glass slides by using an unbalanced magnetron sputtering process in an argon (Ar) atmosphere. The base pressure before deposition was less than 2×10^{-3} Pa. Before deposition, the substrates were etched for 20 min at a substrate bias potential of −800 V in Ar plasma. To enhance film adhesion, Ta ion bombardment (Ta cathode power = 50 W and bias voltage = −600 V) was applied before the deposition. After etching, the Ta coating was deposited with the cathode power of the Ta target set to 200 W, and a bias voltage of −40 V in pulsed DC mode at a frequency of 100 kHz was used. With the flow rate of Ar fixed at 75 sccm, the chamber maintained an Ar pressure of 0.6 Pa. For the deposition of TaC/a-C coatings, high-purity tantalum (Ta) and graphite targets (99.99 at.%) were arranged in conjunction with depositing the composite coatings. Each target had a diameter of 50 mm and was tilted at 45° relative to the substrate. The target and substrate were separated by 60 mm, and the samples were placed on a rotational substrate holder for the deposition. The composite TaC/a-C coatings were deposited by driving the cathodes in pulsed DC mode at a frequency of 20 kHz and a duty of 80% by using Advanced Energy MDX power supplies with SPARCLE pulse units. The cathode power of Ta was maintained at 20 W whereas the cathode power of graphite was set at 200 W. The Ta-C:H coatings were deposited by introducing reactive C_2H_2 (flow rate = 70 sccm), which was ionized and/or dissociated in the magnetron sputtering process. The cathode power of Ta was set at 30 W, and the Ar pressure was maintained at 0.6 Pa. A substrate bias voltage of −40 V was used. The temperature of the sample during the deposition was measured using a thermocouple located near the sample to be within the range of 100 ± 25 °C. The total thickness of the coatings was controlled at approximately 0.6 μm using a deposition time of 20 min.

The chemical-binding characteristics of the deposited films were determined using X-ray photoelectron spectroscopy (XPS; PHI1600) with Mg Kα radiation. The XPS was performed with 2 kV Ar ions to sputter the surface oxide layer for 1 min. Survey spectra in the range from 0 to 1000 eV were recorded for each sample, from which the composition was calculated. The spectral ranges of 25 ± 11 eV and 287 ± 12 eV corresponded to the binding energies of Ta4f and C1s, respectively. The energy was calibrated by reference to the Au $4f_{7/2}$ peak from a clean gold surface at 83.8 eV. The characteristics of the bonding of TaC/a-C and Ta-C:H coatings were also identified using a micro-Raman spectroscope (Ranishaw 2000). The Raman scattering spectra were recorded using a 514 nm Ar laser light source. Using an optical focusing lens, an illuminated spot on the sample surface was focused to obtain a spectral resolution of 1 cm^{-1}. Glancing-angle X-ray diffractometry (XRD; PANalytical X'pert Pro) at a glancing angle of 1° and Cu radiation was used for phase identification. The diffractometer was operated at 40 kV and 30 mA.

2.2. Contact angle measurement

The static contact angle of the deionized water on each sample at room temperature was measured after all Ta-, TaC/a-C, and Ta-C:H coatings were washed alternately in containers with ethanol and deionized water in an ultrasonic cleaner for 30 min each. After samples were dried in a clean dry oven at 55 °C for 6 h, drops generated using a micrometric syringe were deposited onto the surface. The height-to-width ratios of the samples were measured and they were immediately photographed using an instrument for measuring contact angles (FTA-125, First Ten Angstroms, Portsmouth, VA, USA) Each contact angle reported here is the mean of at least 10 independent measurements.

2.3. Surface morphology analyses

The surface morphology of the WS1 human fetal skin fibroblast cells on the deposited coatings was examined using high-resolution field-emission scanning electron microscopy (SEM; JSM-7000F, Joel). Before conducting SEM, a 3-mL aqueous solution containing WS1 cells (1×10^4 cells/mL) was seeded onto the samples and incubated at 37 uC in 5% CO_2 for 48 h. The samples were rinsed with PBS, immersed in distilled deionized water for 10 min, and then dehydrated in an ethanol series (50%, 70%, 90%, 95%, and 100% each for 10 min). The tested samples were then fixed and subsequently dried using critical point drying and coated with platinum.

2.4. Biocompatibility test of MG-63 and WS1 human fetal skin fibroblast cells

The biocompatibility test was performed by individually evaluating the cell viability of MG-63 cells and WS1 cells. Cells were cultured onto the uncoated Ta, TaC/a-C, and Ta-C:H-coated specimens for several days. Cell viability was determined by detecting the intracellular purple formazan that resulted from the yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide). MG-63 and WS1 cells were seeded onto each specimen at a density of 5000 cell/cm^2 and 3.4×10^4 cells/mL and incubated at 37 °C for 5 d. MTT (Sigma-Aldrich) working solution was added to these cells and incubated for 4 h at 37 °C, protecting the cells from light. The purple formazan was eluted using an equal volume of DMSO solution (Sigma-Aldrich) and transferred to a multiwell plate for analysis. The absorbance of the purple formazan was quantified as the optical density (OD) measured at 570 nm using a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) with SoftMax Pro 5.2 241 software (Molecular Devices). All procedures were protected from light irradiation, and the experiments were repeated independently in duplicate.