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Investigation of the antimicrobial properties of modified multilayer diamond-like carbon coatings on 316 stainless steel

Shaun N Robertson [a,b], Des Gibson [b], William G MacKay [a], Stuart Reid [b], Craig Williams [a], Ross Birney [b,*]

[a] Institute of Healthcare, Policy and Practice, School of Health, Nursing and Midwifery, University of the West of Scotland, PA1 2BE Paisley, Scotland, UK
[b] SUPA (Scottish Universities Physics Alliance), Institute of Thin Films, Sensors and Imaging, School of Engineering and Computing, University of the West of Scotland, PA1 2BE Paisley, Scotland, UK

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ABSTRACT

Modified diamond-like carbon (DLC) coatings were deposited onto 25 mm diameter 316 stainless steel discs by pulsed (direct current) hollow cathode plasma enhanced chemical vapour deposition (HC-PECVD). Multilayer films of total thickness 1–2 μm were deposited, both with and without germanium dopant. Characterisation of the coatings was performed by SEM/EDX, surface energy/contact angle analysis, and assessment of possible biofilm-inhibiting properties. Both modified DLC and germanium-doped DLC (Ge-DLC) coatings showed a significant anti-biofouling effect on *P. aeruginosa*, a Gram-negative bacterium. A 90% reduction in *P. aeruginosa* biomass was observed compared to control for both DLC and Ge-DLC, however this effect could not be attributed to germanium incorporation alone. Neither modified DLC nor Ge-DLC showed a significant inhibitory effect on *S. aureus*, a Gram-positive bacterium. Scanning electron microscopy of *P. aeruginosa* biofilms on Ge-DLC coated 316 stainless steel clearly displayed disruption of the cellular wall, as well as leakage of cellular components; this effect was not observed with modified DLC coating. This suggests that germanium-doped DLC coatings may potentially exhibit a cidal mode of action versus *P. aeruginosa* biofilms.

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1. Introduction

Microbial biofilms are ubiquitous in aqueous environments, including but not limited to pipelines, food & beverage industry, ship hulls and heat exchangers; in fact, biofouling may occur on virtually any surface in contact with water [1,2]. With biofilm formation on such surfaces there are significant implications. For example, internal diameter is reduced in pipes, leading to reduced flow rates; microbial influenced corrosion of metals [3], leading to damage and subsequently necessitating the replacement of pipelines; and increased drag of ships, leading to higher fuel consumption with the associated environmental impacts [4]. Notably, microbial biofilms have the potential to act as reservoirs of infection in potable and washing water systems, which can have a devastating impact on community [5,6] and healthcare related environments [7].

A biofilm can briefly be described as a consortium of cells, encapsulated within an extracellular polymeric substance (EPS) [8,9]. The EPS provides them with a high degree of hydration, and strong attachment to a surface when compared to the initial planktonic cell attachments preceding biofilm formation; and, in turn, increased resistance to removal via cleaning [10]. For example, chlorine may prove to be ineffective in treating established biofilms [11,12].

Methods to prevent microbial attachment, through the development of antimicrobial coatings for industrial and clinical settings, are highly sought after in order to combat the increasing costs and morbidity/mortality that can be associated with biofilms. Diamond-like carbons (DLCs) have found utility in a wide range of applications, perhaps most notably as protective coatings for metals against corrosion [13–16]. DLCs represent a class of amorphous carbon materials; the main subtypes being amorphous hydrogen-carbon alloy (a-C:H), hydrogen-free amorphous carbons (a-C) and tetrahedrally-structured amorphous carbons and their hydrogenated analogues (ta-C and ta-C:H), which can contain in excess of 90% C—C sp^3 bonding. Previous research into silicon-doped DLC has shown reduction in biofouling, with the common Gram-negative bacteria *Pseudomonas aeruginosa* and Gram-positive bacteria *S. aureus* and *S. epidermidis* [17,18]. Doping of DLC with known antimicrobial metals such as copper [19], silver, and platinum [20], has been undertaken, but this often involves the incorporation of metallic nanoparticles in and on the upper layer of the DLC. Recent research has demonstrated that nanoparticles (NPs) have even greater cytotoxicity than non-nanoparticle forms of such metals [21–24], potentially precluding their use in healthcare and environmental applications, if a suitable concentration window cannot be achieved. Research has also shown that ion release from NPs incorporated into DLC coatings is dependent on nanoparticle

* Corresponding author.
E-mail addresses: Shaun.Robertson@uws.ac.uk (S.N. Robertson), Des.Gibson@uws.ac.uk (D. Gibson), W.mackay@uws.ac.uk (W.G. MacKay), Stuart.Reid@uws.ac.uk (S. Reid), Craig.Williams@uws.ac.uk (C. Williams), Ross.Birney@uws.ac.uk (R. Birney).

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size, with increasing ion release being related to decreasing NP size, which allows tailoring of the antibacterial and toxicity windows for medical implants [25]. An additional problem highlighted in prior literature on the anti-biofouling effects of doped DLC is the common use of colony-forming unit (CFU) tests to test the coatings' properties. These tests are often performed by dropping small quantities of an inoculum onto a surface, either in sterile water or phosphate buffered saline (PBS) solution. However, the planktonic minimum inhibitory concentrations (MIC) can differ markedly versus the minimum biofilm eradication concentration (MBEC). This is clearly evidenced in the clinical setting [26], with antimicrobial drugs often having MBECs that can be up to 100 times greater than the MIC; in some cases, they are completely ineffective. This observation is not limited to conventional antimicrobials, with concentrations of silver required to eradicate mature *P. aeruginosa* biofilms being 10–100 times greater than the MIC of planktonic cultures [27].

Germanium is a metalloid from the carbon group, and is chemically similar to silicon. Elemental germanium in suspension has been shown to have antimicrobial properties versus *P. aeruginosa* and *Staphylococcus aureus* in both planktonic (MIC = 6.25 mg/ml) and biofilm (MBEC 25 & 50 mg/ml respectively) [28]. In this work, a hollow cathode plasma enhanced chemical vapour deposition (HC-PECVD) system (Sub-One Systems, Tucson, AZ), specifically designed for coating interior pipe surfaces, was used to deposit multi-layer modified DLC coatings and germanium-doped multi-layer DLC (Ge-DLC) coatings; the optimized DLC and Ge-DLC were then evaluated to assess for any biofilm-inhibiting and antimicrobial properties.

2. Materials and methods

2.1. Preparation of DLC and Ge-DLC

Multilayer modified DLC and Ge-DLC coatings were deposited onto 25 mm diameter 316 stainless steel discs by pulsed-DC hollow cathode plasma enhanced vapour deposition (HC-PECVD) deposition as previously described elsewhere [29,30]. However, in this current work, an aluminium stage (Fig. 1) was fabricated; designed to conform to the interior of a 4″ pipe (cathode) and to accommodate planar substrates. This stage ensured that the substrates remained in electrical contact with the cathode; this was necessary to enable film deposition on the stainless steel discs. SS316 and silicon wafer witness substrates were initially cleaned with acetone and lint free cloth to ensure a contaminant-free surface and a base pressure of 1×10^{-3} Torr was attained in the chamber prior to deposition. A hydrogen and argon plasma substrate pre-heating step was performed, followed by an argon-only sputter etch, which has previously been shown to enhance adhesion of multilayer DLC [2,31,32].

Both the DLC and Ge-DLC coatings were 5-layer graded designs deposited with argon as the working gas, and using tetramethylsilane (TMS, $Si(CH_3)_4$), acetylene (C_2H_2) and, in the case of the Ge-doped coating, finally tetramethylgermane (TMGe, $Ge(CH_3)_4$) in the top layer. These coatings were deposited in a multi-stage process, beginning with TMS, which results in deposition of an amorphous SiC:H adhesion layer; with increasing fraction of acetylene and decreasing fraction of TMS in the following layer steps, terminating with either an a-C:H or Ge-doped a-C:H top layer.

The deposition processes are summarized in Table 1.

2.2. Characterisation of DLC and Ge-DLC coatings

2.2.1. SEM and EDX analysis

Characterisation of the multilayer modified DLC and Ge-DLC coatings was performed by scanning electron microscope (SEM) analysis of both the surface and cross-section of coated silicon wafer witness pieces. Analysis was performed on a Hitachi S-4100 scanning electron microscope at 10 kV acceleration voltage. Composition of the upper layers of the DLC thin film was determined by energy dispersive X-ray (EDX) analysis of the witness piece on an Oxford instruments X-Max 80 detector at three acceleration voltages (10, 15 & 20 kV); this allows elemental composition to be determined at varying depths within the multilayer sample.

2.2.2. Surface roughness characterisation

Average surface roughness measurements were performed on a Dektak 3ST surface profilometer (Veeco, USA). Surface roughness scans were performed in triplicate on each sample, with the following measurement parameters: scan length 2000 μm (1000 data points over scan length), 30 mg force. Analysis was performed in triplicate to determine the R_a (average roughness), R_p (maximum peak height) and R_v (maximum valley depth).

2.2.3. Contact angle and surface energy

Contact angle measurements were performed by the sessile drop method with a CAM200 contact angle goniometer and SFECAM software (KSV Instruments, UK). Surface free energy (SFE) was calculated by use of the Fowkes method [33], which requires the contact angle

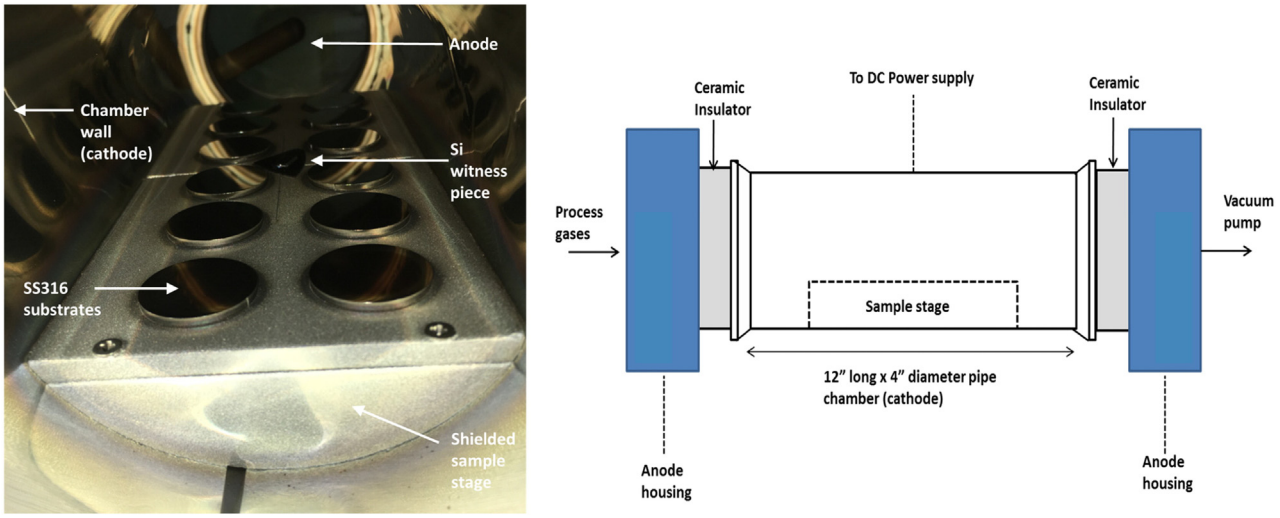

Fig. 1. Sample arrangement in chamber and schematic of deposition system. An aluminium stage was fabricated to conform to the internal diameter of a 4″ pipe. Left image shows substrates (SS316 with Si witness piece) loaded on the aluminium stage and aluminium foil lining of the pipe chamber. Gas entry head anode is visible in background. Right image shows simplified schematic of deposition apparatus.

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