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The effect of sterilization processes on the bioadhesive properties and surface chemistry of a plasma-polymerized polyethylene glycol film: XPS characterization and L929 cell proliferation tests

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Abstract

The influence of several sterilization processes (autoclaving, γ-ray irradiation, ethylene oxide exposure and Ar/H_2 low pressure plasma treatment) on the surface chemistry and the bioadhesive properties of thin films (thickness ~20 nm) of plasma-polymerized diethylene glycol dimethyl ether has been studied. X-ray photoelectron spectroscopy (XPS) analysis and cell proliferation tests were used to characterize the surfaces. The XPS results revealed in all cases a change in the surface chemistry of the layer after sterilization, whereas the conservation of non-bioadhesive properties of the coating depends on the type of sterilization process. In particular, the low pressure plasma-based sterilization technique leads to a loss of the non-bioadhesive properties of the plasma coating, whereas the coatings are resistant to the other standard decontamination techniques. This property makes them suitable for biomedical applications, provided that an appropriate sterilization process is selected.

Keywords: Plasma polymer; Sterilization; Cell adhesion; Anti-adhesive surface; XPS

1. Introduction

Understanding and controlling biointerfacial phenomena are crucial in various applications, such as biosensing, biological testing and production of medical tools (instruments, accessories and implants). Particularly in the latter case, non-bioadhesive coatings are desired for the biological integration of these objects by limiting their interactions with physiological fluids [1,2]. Precise control of the interactions between material surfaces and the biological milieu is important particularly when the interface must prevent attachment of microorganisms that can lead to biofilm formation. Among the strategies designed for the prevention of biofilm formation, poly(ethylene glycol) (PEG) is a promising compound that can be used both for improving biocompatibility and for reducing bacterial adhesion [3–7].

PEG (also referred to as PEO: poly(ethylene oxide)) is a polymer composed of $–(CH_2–CH_2–O)–$ repeat units. Of the many models proposed in order to explain its anti-bioadhesive properties, steric stabilization and an excluded-volume effect are the most commonly cited [8]. Due to its exceptional properties, several approaches have been developed to produce PEO layers, e.g. physical adsorption [9–12], radiation and chemical cross-linking [13,14], deposition of self-assembled monolayers [15], spin coating stabilized by ion beam treatment [16], covalent immobilization [17], chemisorption of PEO-thiol on gold surfaces [18], or plasma polymerization of *n*-glyme [19] or diethylene glycol dimethyl ether (DEGDME) [20,21]. Plasma polymerization

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of DEGDME, for instance, allows the fabrication of thin coatings (<50 nm) with good adhesion on a large variety of substrates without affecting the bulk properties of the initial material, which is of crucial importance in the case of implants. Moreover, the deposition can be done in one step and can also be performed on 3D surfaces.

If this material is to be used as a coating layer on implants or on tools for surgery, it has to withstand sterilization processes. According to existing guidelines, all devices that are intended to penetrate the human body and come into direct contact with the patient's first immune defence system and tissues have to be sterilized in order to prevent infections caused by pathogenic microorganisms. In order to stress the importance of the sterilization step, it should be noted that bacterial contamination of biomaterial surfaces during surgery is the primary cause of implant-related infections [22]. However, previous studies have shown that sterilization can affect the surface properties of polymers [23–25]. It is therefore of paramount importance to know whether sterilization processes change the surface chemistry and/or affect the bioadhesive properties of thin plasma polymerized PEO coatings.

In this study, we have investigated surface chemical modifications and changes in the bioadhesive properties of plasma deposited PEO coatings with a thickness of ~20 nm obtained by plasma polymerization of DEGDME after application of the most common sterilization and decontamination processes: autoclaving, ethylene oxide (ETO) treatment and γ-ray irradiation, together with the application of two types of Ar/H_2 low pressure plasma treatments that can be used to destroy both bacterial spores [25] or to inactivate bacterial endotoxins [26,27]. The chemical modifications of the surface induced by these techniques were analysed by X-ray photoelectron spectroscopy (XPS), whereas the bioadhesive properties were studied using L929 fibroblast cells as model.

2. Materials and methods

2.1. Substrate

In this study, all PEO deposition was done on Thermanox slide surfaces (Nunc company). This material was selected because it is a transparent polymer, which thus allows the direct observation of cells, and is temperature resistant from −70 to +150 °C. Moreover, Thermanox slides are treated on one side for enhanced cell attachment and growth and therefore can also be used as positive control for cell attachment and proliferation. For the ellipsometry measurements, one side of polished silicon wafers (1 1 1) were used as substrates.

2.2. Poly(ethylene oxide)-like layer deposition

The deposition conditions of the plasma polymerized poly(ethylene oxide) (PEO-like) films are described in detail elsewhere [28]. Briefly, the system used was a reactor in stainless steel (dimensions 300 mm × 300 mm × 150 mm) with two symmetrical internal parallel-plate electrodes (diameter of electrodes = 140 mm, distance between the two electrodes = 50 mm). The plasma was generated by a radio frequency generator (13.56 MHz) connected to the upper electrode, with the lower electrode grounded and used as the sample holder. The plasma polymerization was carried out by using a pulsed plasma discharge (time on = 10 ms, time off = 100 ms, nominal power = 5 W) of pure DEGDME ($(CH_3OCH_2CH_2)_2O$, from Sigma–Aldrich, used as received). Ellipsometry measurements showed that the films produced are uniform, with a thickness of 23 ± 0.5 nm.

2.3. Sterilization processes

2.3.1. Autoclaving

Untreated and PEG-coated Thermanox slides were packed in aluminium foil and autoclaved for 12 min at 134 °C, the suggested autoclaving program for wrapped instruments, in a Euronda, E4 Plus-18 autoclave. After cooling, the samples were subjected to the cell culture adhesion and proliferation test by putting them into 24-well plates, adding 400 μl of the cell suspension calibrated to 5×10^4 cells ml^{-1} and observing them every 24 h.

2.3.2. ETO sterilization

Untreated and PEG-coated Thermanox slides were packed in Tyvek pouches, sealed and sent for ETO sterilization treatment. The reaction occurs at 50 °C, under ETO vapour, for 3–4 h. Forced gas extraction for 3 days was then performed at 42 °C in a confined environment. The samples were kept in the sealed pouches until use.

2.3.3. γ-ray irradiation

Untreated and PEG-coated Thermanox slides were packed in Tyvek pouches, as an underlayer, and then aluminium pouches, as an external layer, sealed and sent for sterilization via γ irradiation. The reaction occurs at room temperature, with a 25 kGy γ irradiation dose. The samples were kept in the sealed pouches until being used.

2.3.4. Plasma treatments (Ar/H_2 plasma)

Plasma-based sterilization and decontamination is generally via one of two main pathways. The first is based on the physical removal of pathogens by extensive etching or sputtering. This is commonly achieved by exposing treated objects to the active plasma discharges, providing sufficient fluxes of chemically active particles and ions that are capable to gradually sputter or volatilize treated organic materials. However, high etching rates induce the risk of degradation of the treated objects, which is particularly so in the case of polymer-based materials whose properties can be affected by extensive etching (e.g. [29]). The second pathway relies on the modifications of the structural or chemical properties of the treated substances, which results in suppression of their biological

ID	Title	Pages
1747	The effect of sterilization processes on the bioadhesive properties and surface chemistry of a plasma-polymerized polyethylene glycol film: XPS characterization and L929 cell proliferation tests	7