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Ultra-thin polymer coating for promoting neural cells integration with neural implants

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ABSTRACT

Human neuroblastoma (SH-SY5Y) cells were used to demonstrate biocompatibility (including control over direction of neurites) for multilayer thin polymer coatings with neural cells. The ultra-thin (<20 nm) coatings were synthesised using plasma deposition process with (CH_4, C_2H_2 and O_2) gasses as precursors. The aim is to use these thin films to add bio-selectivity to orthopaedic and neurological implants such as cervical disc and interbody replacement, in addition to providing other qualities such as degradation resistance and permeation barrier. X-ray photo electron spectroscopy and contact angle measurements were used to provide surface molecular and surface energy information, respectively. The thin coatings' functionality remained intact despite robust sterilisation process. The coatings showed no toxicity effects on neural cells growth and spreading after 6 days. Neural cells responded favourably (cell growth and spreading) to thinner (<6 nm) coatings that are rich in oxygen. Closer inspection revealed that neurites grow denser on surfaces with C=O functional groups and reacted negatively to the increase in C–O functional groups on the surface. Best neurite-like differentiation towards neural-like cells was observed on surfaces that were plasma deposited using C_2H_2 gas (with or without oxygen). Plasma treatment using CH_4 produced thin coatings with lowest surface energy that repelled neurites cells (very low cell density). Hence, the latter treatment is useful for cases where cells/bio-coatings inhibition is desired.

Introduction

Neural implants are devices used to totally or partially restore neural based functionality like sensory and motor function after injury or genetic disease. For neural implants to be successful, the neural tissue must be fully integrated with the corresponding stimuli, such as wires, electrodes and connectors and other components [1,2]. The interaction between neural tissue and the active implants at the interfaces dictate the degree of implants success, to large extent, on achieving its intended purpose [3–5]. The long term safety and effectiveness of metal based medical implants are under scrutiny by the scientific community after the discovery of numerous associated medical complications [6–11].

There is a significant gap in the current knowledge regarding the proper material to be used for active medical implants' integration and long-time functioning in the human body. There is a consensus among researchers in the medical implants field that one material cannot provide all the signals necessary for complex neural tissue integration, as well as having anti-repellent properties for undesired bacteria or blood constituents [12–18]. Ultrathin polymer coatings are introduced in this project to add multiple functionalities to implants made from polymers such as PEEK or metals such as Titanium or Stainless Steel.

Bio selectivity is an important objective to be achieved for the integration of medical implants in which a selective tissue attachment must be ensured [19,20]. Thin coatings were used previously for improving the biocompatibility of implants either with bone [19,21–23] or neural cells [24,25], but not both. There are other studies that showed enhanced surfaces with multiple bio functionalities that supported the growth of one type of cells like osteoblast with bactericidal functionality [20] However, bio selectivity is still a complex issue in which majority of synthesised coatings often supported the growth of one type of cells and not the others. In our preliminary work, we discovered the remarkable adhesion strength of functionalised plasma deposited ultra-thin organic coatings (less than 20 nm thin) to polymers [26–30] and to osteoblast-like cells [31]. We deposited an oxygen rich thin layer from plasma containing methane and oxygen (CH_4/O_2) onto PEEK polymer coatings. The potential for forming strong covalent

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bonding in this case was further enhanced in the depositing layer by subjecting it to ion bombardment from the plasma using plasma immersion ion implantation technique. Furthermore, the synthesised single layer showed high surface energy and oxygen functionality for strong molecular bonding [26]. Osteoblast like cells showed strong response to plasma deposited thin polymer coatings with high amount of oxygen functionalities. The response was weak for both nitrogen functionalities and non-functional (non-polar) surfaces. Nitrogen functionalised surface repelled the cells into creating thicker clusters with poor spread [31].

Proper synthesising control of these ultra-thin coatings and understanding of structure–property relationship provide a great potential for different but complementary qualities to a material without a significant change in dimensional integrity. Molecular chain conformation and dynamics, play an important role not only in fabrication but also in the functionalisation of numerous polymeric nano-structured materials.

Neuroblastoma cell line SK-N-SH has been used previously for multiple objectives including in cytotoxicity assays and as a transfection host [32,33]. This cell line was established in 1970 and originated from metastatic bone tumour from a 4 years old female [32]. The growth patterns of neuroblastoma cells often follow two stages, initially they grow as clusters forming clumps. At a later stage, surface conditions allowing, they form neurites that can also develop into more complex neural structures with defined components such as dendrites or dendrons and an axon or axis cylinder or neuraxon [34–36].

In this project, we introduce a series of ultra-thin polymer coatings, some of which we used successfully in improving adhesion to osteoblast cells, to be used for improving adhesion and compatibility with neural cells. The emphasis is drawn at coatings thinner than 20 nm to avoid delamination, and swelling in wet environment. The aim is to provide bio selectivity option using one type of thin coating for medical implants to achieve better adhesion and biocompatibility with osteoblast and neural cells.

Experimental

Synthesis of thin coatings by plasma deposition

The plasma deposition equipment that was used in this study consisted of a radiofrequency (13.56 MHz) power supply, capacitively coupled via an impedance matching network to a plasma treatment chamber (450 mm in diameter) using an externally mounted antenna. The sample holder consisted of a stainless steel plate mounted on a glass tube, which is electrically isolated from the chamber and biased to a negative voltage. The base pressure of the chamber was 7×10^{-3} Pa and the operating pressure range was from 0.63 to 0.66 Pa. The flow rates of gases were 40 sccm. The operating power of the RF power supply was 200 W, with 25–50 W reflected power. The sample holder was connected to a pulsed power supply delivering direct current (DC) pulses of 10 kV at a frequency of 2000 Hz with a pulse duration of 25 μs. PIIID plasma treatments of Si surfaces using different gases were performed with a bias voltage of 10 kV. The experimental runs are shown in Table 1. Denomination such as CH_4/O_2 means that CH_4 and O_2 gases were used in the plasma treatment run with equal concentration. While $CH_4 + O_2$ means two sequential plasma treatment processes that involves CH_4 only in the first stage then O_2 only in the second stage.

XPS

X-ray photoelectron spectra were acquired for the plasma treated samples and also for the untreated control. A Kratos Axis DLD Ultra operated in spectral mode was employed to analyse the samples. 1250–0 eV wide scans were acquired in low energy resolution with a pass energy of 160 eV while core-line spectra were acquired in high resolution conditions with a pass energy of 40 eV. The XPS instrument is equipped with a flooding electron gun coaxial to the analyser axis making the compensation particularly efficient. Compensation conditions were set in order to maximise peak intensity and reduce the full-width at half maximum (FWHM) leading to an energy resolution of ~0.35 eV. Energy alignment of the spectra was performed taking the CH_x component of the carbon 1 s core line at 285 eV. Core line analysis was performed with a software based on the R platform (The R Project for Statistical Computing, http://www.r-project.org/). Linear background and Gaussian components were used to perform the peak fitting. Quantifications were carried out utilising the sensitivity factors provided by the instrument manufacturer.

Table 1
Plasma deposition process parameters including the precursor gas(es), gas flow rate and plasma treatment time.

Sample/treatment gas	Gas flow (CCM)	Plasma treatment time
CH_4	40	15 min
C_2H_2	40	15 min
CH_4/O_2 (1:1)	40	15 min
C_2H_2/O_2 (1:1)	40	15 min
$(CH_4 + O_2)$	40/40	7.5 + 7.5 min
$(CH_4/O_2 + O_2)$	(20 + 20)/40	7.5 + 7.5 min
$(O_2 + CH_4)$	40/40	7.5 + 7.5 min
$(CH_4/O_2 + CH_4)$	(20 + 20)/40	7.5 + 7.5 min
$(C_2H_2 + O_2)$	40/40	7.5 + 7.5 min

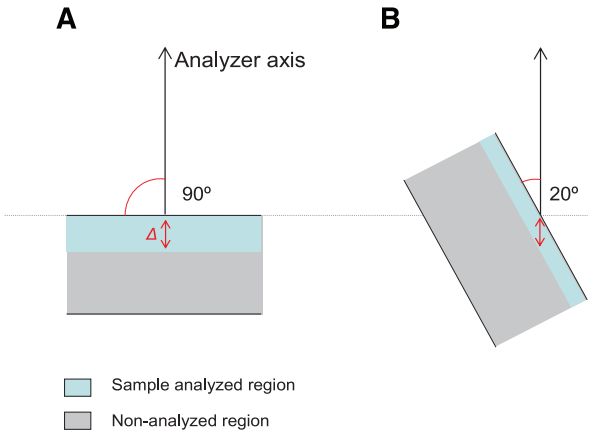

Fig. 1. Effect of the tilt on the X-sampling depth. In normal conditions, (A) the analyser axis is orthogonal to the sample surface. Only photoelectrons traveling along the analyser axis direction can be detected. As a consequence the thickness of the analysed region corresponds to the sampling depth Δ. In tilted conditions (B) the thickness of the analysed region decreases as a function of the tilt angle.

Evaluation of the coating thicknesses by angle resolved XPS

Angle resolved XPS was utilised to estimate the plasma deposited coatings' thickness. Coating thickness was computed from the peak intensity of elements belonging to the sample surface and to the substrate. The thickness of the coating was estimated via the intensity ratio of C1s and Si2p peaks measured at different tilt angles. For this purpose, angle resolved XPS measurements at tilt angle of 90° (sample normal to the analyser axis) and tilt angle of 20° were performed (see Fig. 1).

In brief, the method relies on the decay of the photoelectron signal intensity coming from the substrate (silicon peak intensity) due to the presence of the thin polymer coating. The higher the thickness of the polymer coating, the higher the decrease of the silicon core line intensity. Fig. 2 compares the wide XPS spectra of