Osteoblast Activity on Teflon after Ion Implantation
Silk Fibroin Scaffolds for Tissue Engineering
Multi-Wall Carbon Nanotubes for Lipase Immobilization
Surface Structures and Osteoblast Activity on Biomedical Polytetrafluoroethylene Treated by Long-Pulse, High-Frequency Oxygen Plasma Immersion Ion Implantation**

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Polytetrafluoroethylene (PTFE) is a biologically safe polymer used widely in clinical medicine including oral and orthopedic surgery. However, the high bio-inertness of PTFE has hampered wider applications in the biomedical fields. In this work, we extend the treatment time in long-pulse, high-frequency oxygen plasma immersion ion implantation of PTFE and a more superhydrophobic surface with a water contact angle of 160°° is created. X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) reveal that the optimized long-pulse, high-frequency oxygen plasma immersion ion implantation process induces a rougher surface and to a lesser extent alters the surface oxygen concentration on the PTFE. Our data, especially long-term contact angles, suggest that the superhydrophobicity stems from surface roughness alteration. Furthermore, the activity of MC3T3-E1 osteoblasts cultured on the treated surfaces is promoted in terms of quantities and morphology.

Polytetrafluoroethylene (PTFE) is a semi-crystalline polymer with a smooth linear molecular profile \((-\text{CF}_2-\text{CF}_2-)_n\), where C stands for carbon atoms and F stands for fluorine atoms. It is used in a variety of applications because of its high temperature stability, excellent chemical resistance, low dielectric constant, high electric resistance, very low surface free energy, and friction coefficient. However, its low surface free energy and poor adhesion have hampered many applications such as biomedical engineering. The inherent chemical inertness of PTFE also renders chemical surface modification difficult and direct metallization and adhesion to other materials difficult, if not impossible. This has further limited the use of PTFE in many commercial applications.

PTFE can be expanded to porous ePTFE which generally has good biocompatibility because of its higher chemical stability. In addition, the materials are non-toxic, do not leave residues, and do not degrade in vivo. As a result, ePTFE is widely used in clinical medicine, for instance, being one of the most reliable materials in implants and devices with proven biological safety. However, the materials are typically classified as bio-inert due to its very high hydrophobic nature and lack of functional groups to interact with the cellular environment.

In order to enhance the bioactivity and biocompatibility of ePTFE, surface modification is a possible means. The surface hydrophobicity and cytocompatibility can be enhanced if surface treatment is conducted properly. Chemical and physical methods such as radiation induced grafting, ultraviolet or vacuum ultraviolet, plasma or plasma grafted treatment, and ion implantation have been proposed. Plasma treatment and ion implantation have been used to modify the wettability, surface energy, mechanical strength, adhesion strength, wear resistance, electrical conductivity, and other properties. Ion implantation is attractive because of its flexibility, effectiveness, and environmental friendliness. Furthermore, it does not affect the bulk properties as the effects are predominantly surface. Plasma modification is also advantageous since no toxic organic
solvents are typically released into the environment and main instrumental parameters such as current, pressure, and voltage are easy to control and reproduce. Moreover, plasma surface modification can be carried out at a low temperature which bodes well for temperature sensitive polymers.[18]

Plasma treatment and ion implantation can be combined in plasma immersion ion implantation (PIII).[19] When a polymer is treated by PIII, both plasma surface modification and ion implantation effects can be attained. Long-pulse, high-frequency quasi direct-current (DC) oxygen PIII has been utilized in our laboratory to create a super-hydrophobic PTFE surface with a water contact angle of over 150°.[19] Scanning electron microscopy reveals submicrometer–nanometer structures on the PTFE surface after this new plasma treatment which combines the merits of plasma surface modification and energetic ion bombardment. In comparison, plasma surface modification is the dominant effect in conventional short-pulse, low frequency PIII.[19] Cell viability assay, alkaline phosphatase (ALP) activity test, and real-time polymerase chain reaction (PCR) analysis have been performed to investigate the osteoblast behaviors.[20] Osteoblastic cells are used to study bone metabolism and biomaterials/cell interactions essential to bone tissue engineering. MC3T3-E1, which is a strain of tissue culture cells derived from mice, is one of the most convenient and physiologically relevant systems to study the interaction between osteoblast cells and biomaterials.[21] Our previous results disclose that all three modification techniques, namely long-pulse, high-frequency PIII, short-pulse, low-frequency PIII, and simple plasma exposure can all promote osteoblast cell adhesion and proliferation. Moreover, improvement in the ALP, osteopontin (OPN), and osteonectin (ON) expression of the seeded osteoblasts can be achieved. However, among the three treatments, only long-pulse, high-frequency O₂ PIII can promote the osteocalcin (OCN) expression of osteoblasts.

Our previous study thus suggests that there is room for improvement in the treatment protocol. That is, the conditions of long-pulse, high frequency PIII in the treatment the PTFE surface must be further optimized and the associated effects on osteoblasts must be investigated in more details. In this paper, we report in details the effects of long-pulse, high-frequency PIII on PTFE. As the treating time is extended to 1 h, contact angle measurement, X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM) disclose changes on the polymer surface. In order to investigate the surface cytocompatibility of the altered surface, the growth, and morphology of the seeded osteoblasts on the treated and untreated PTFE are assessed.

Materials and Methods

Preparation of PTFE Samples

PTFE purchased from Good Fellow with a thickness of 0.25 mm cut into 7 cm by 8 cm pieces was processed in the Plasma Laboratory of City University of Hong Kong.[22] To conduct high-frequency (500 Hz) and long-pulse (200 μs) oxygen PIII, the high voltage sample stage and supporting voltage feedthrough rod were shielded from the plasma by a metal cage made of aluminum.[19] A hole with a radius of 100 mm was made in the center of the top cover. An aluminum mask with a square opening of 6 cm by 7 cm was covered by a stainless steel mesh [120 mesh per 2.5 cm (1 inch) and 65.0 μm (0.0026 inch) wire diameter]. The PTFE sample was placed 1.0 cm from the mask. The oxygen flow rate of 6.0 sccm, negative bias of −5 kV, and RF power of 1000 W in these experiments were similar to the ones adopted in our previous studies.[19,20] Voltage pulses of −5 kV were applied to the sample stage for 1 h. The schematic of the setup is depicted in Figure 1. It should be noted that although the substrate holder was metallic and electrically conductive, the PTFE samples were dielectric materials. Simulations and experiments showed that the surface potential would be gradually reduced due to surface charging.[23,24] Hence, the full negative surface potential might not be maintained throughout the 200 μs pulsing. Surface charging may be an important factor in the modification efficacy of insulators such as PTFE and more work is needed to investigate this issue.

Surface Characterization

After the long-pulse, high-frequency PIII treatment was conducted on the PTFE, static contact angle measurements using distilled water as the medium were performed on a Ramé-Hart (USA) instrument at room temperature. The surface contact angles were measured at various time
intervals to study the hydrophilic recovery. Each data point represents the average of five measurements conducted on different parts of each specimen for statistical accountability.

The elemental depth profiles and chemical states were determined by XPS on a Physical Electronics PHI 5802. A monochromatic aluminum X-ray source in concert with argon ion sputtering was used to determine the elemental depth profiles. A sputtering rate of 0.54 nm/0.5 min derived from silicon oxide under similar conditions was used in the depth calibration. The step size and the constant pass energy of XPS was 0.1 and 11.75 eV, respectively. The surface morphology was evaluated by a tapping mode AFM (NanoScope V MultiMode System, Veeco). All the measurements were performed under ambient conditions and the scanned area on each sample was 50 μm × 50 μm. As a standard protocol, all the samples were ultrasonically cleaned in absolute ethanol and DI water for 5 min sequentially before conducting AFM.

Cell Culture

MC3T3-E1 cells provided by The University of Hong Kong were grown in a Dulbecco’s Modified Eagle Medium (DMEM, Invitrogen Cat no.12430047) with 10% fetal calf serum (FCS, Hyclone Cat no. SV 30087.02) and 1% pen strep glutamine (Gibco 10378). The cultures were maintained at 37 °C under 95% air and 5% CO2. The culture medium was changed every 3 days until the cells reached 80–100% confluence. Each specimen was sterilized in 75% ethanol and washed twice with phosphate buffered saline (PBS) and then placed on a 35 mm dish. The MC3T3-E1 cells were seeded onto the samples at a concentration of 10,000 cm⁻² in the complete medium.

Cell Adhesion and Proliferation

MC3T3-E1 cells were seeded onto the sample surface. After 2 and 6 h of incubation, they were rinsed twice with PBS to remove weakly attached and unattached cells. They were then fixed with 2% paraformaldehyde and stained with Hoechst 33342. Cells cultured for 7 days on the treated and untreated PTFE specimens were retrieved by trypsinization. The trypsinized cells of each specimen were counted by a hemocytometer. Three independent experiments were performed to improve the statistics and the data were analyzed by the Student’s two-tailed t-test.

Cell Morphology

The morphology of the MC3T3-E1 cells cultured on the treated and untreated PTFE were observed by fluorescence staining. After 7 days of growth, the samples were rinsed with PBS, fixed with 2% paraformaldehyde. Subsequently, the cytoskeleton protein f-actin were stained with phalloidin-fluorescein isothiocyanate (Sigma) and the nuclei were counterstained with Hoechst33342 (Sigma). The pictures were taken by a digital camera (Carl Zeiss Axio Observer Z1).

Results and Discussion

Aging of PTFE

The PTFE surface is modified by the oxygen plasma treatment. Figure 2 shows the water contact angles versus aging time observed from the treated and control pristine samples. A highly hydrophobic surface is obtained right after the PIII treatment. The initial contact angle is about 160.28°, but this value diminishes with time eventually stabilizing at about 148° after 30 days. In comparison, the surface contact angle on the untreated PTFE control is about 120° and steady with time. The surface contact angles have been reported to vary between 102.5° and 130.0°.[25] The increase in the contact angle values after long-pulse, high-frequency oxygen PIII suggests the formation of hydrophobic groups on the treated polymer surface and/or altered surface topography.[26] According to previous investigations, the water contact angles measured on most polymers after simple plasma exposure tend to return to the original values. However, after ion implantation, the water contact angle has been observed to stabilize without reverting back to its original value.[27] It is acknowledged that physical change can be stably established on the polymer surface for a long time. After the long-pulse, high-frequency PIII treatment, the stable water contact angle on the PTFE samples of 148° suggests physical surface alteration after this treatment.

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<th>Table 1. Atomic percentages and elemental ratios determined from untreated and O₂ PIII PTFE.</th>
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Fig. 2. Water surface contact angles versus aging time.
Chemical Surface Composition

The change in the surface chemical composition induced by long-pulse, high-frequency oxygen PIII is evaluated by XPS. The elemental compositions and elemental ratios are summarized in Table 1. The untreated PTFE has an O/C ratio of 0.035. After PIII, the O/C atomic ratio increases slightly to 0.047. A low concentration of O (1.24%) detected on the untreated PTFE samples. Based on our previous analysis, after half an hour of oxygen plasma exposure and short-pulse,
low-frequency PIII, the O/C atomic ratio can increase to 12.8%.[19] The slightly increased O content (1.74%) after 1 h long-pulse, high-frequency O₂ PIII treatment implies that the plasma effect is not predominant. Moreover, it is obvious that the O/C ratio of PTFE surface after 1 h long-pulse, high-frequency O₂ PIII is even less than that ratio of after treatment for half an hour.[19]

To further evaluate the changes after long-pulse, high-frequency oxygen PIII, the C1s and O1s high-resolution scans are fitted and analyzed. Figure 3(a–c) depicts the typical C1s, O1s, and F1s XPS high-resolution spectra acquired from the O₂ PIII PTFE samples. It can be observed that O₂ PIII produces oxygen containing functional groups such as C–O and C=O in the surface region and some CF₂/C=O is also formed. Although hydrophilic C–O and C=O bonds are formed, their concentrations are quite low and they may not constitute the main reason for the super-hydrophobic PTFE surface with a water contact angle of over 160°. The depth profiles of C, O, and F in the treated and untreated PTFE are displayed in Figure 3(d) and (e). The intensities of all these elements do not show obvious changes before and after PIII treatment, thereby suggesting that the oxygen ions are not implanted.

Surface Topography

Surface roughness is another important factor impacting cell behavior[28] and AFM is utilized to determine the surface morphology. The surface roughness values are determined from regions of 50 μm by 50 μm and the images acquired from the pristine and O₂ PIII PTFE are shown in Figure 4. A striated and asperous surface with sharp rods with sizes of several hundred nanometers is detected on O₂ PIII PTFE. In comparison, although the morphology of the untreated PTFE surface is also somewhat striated, it is relatively smooth. The sizes of these rods range from several hundreds nanometer to 1 or 2 μm. The surface of this O₂ PIII PTFE is even rougher than that of PTFE treated in the same way for only half an hour.[19] Therefore, less oxygen groups but rougher morphology are induced on the PTFE after 1 h long-pulse, high-frequency O₂ PIII than after half an hour the same treatment. As a result, the surface of the treated PTFE is more super-hydrophobic than the L-PTFE previously reported.[19] This change in the surface topography may benefit cell adhesion and the increased surface roughness affects the water contact angles as aforementioned.

Cell Behavior

In order to evaluate the biocompatibility after PIII, MC3T3-E1 cells are employed to characterize cell adhesion and growth on the surface. The results are shown in Figure 5. The MC3T3-E1 cells exhibit different adhesion ability. Although they can adhere onto both samples after 2 h, the O₂ PIII PTFE shows more cell adhesion compared to the control. The proliferation rate in terms of number of cells is also higher on the treated sample than the untreated materials after 6 h of incubation. The data are consistent with our
previous results,\,[20] although the formation of C−O and C=O bonds is compressed after 1h of long-pulse, high-frequency PIII. The promoted osteoblastic cell adhesion ability onto the PIII PTFE samples arises from the increased surface roughness. Our results are consistent with previous reports stating that grossly rough surfaces aid cell adhesion on a nanometric scale.\,[28]

Morphology and Quantity of Cells

The morphology of cells cultured on each specimen after 7 days is shown in Figure 6. The cells are attached and extended on each specimen. The majority of the cells on the untreated PTFE [Fig. 6(a)] fail to spread well although they appear to attach to the substrate surface. In contrast, a large proportion of the cells on the PIII specimen is well attached and spread [Fig. 6(b)]. This provides definitive proof that long-pulse, high-frequency PIII conducted under the proper conditions is a useful technology that benefits growth of osteoblastic cells.

Furthermore, the number of cells cultured on each specimen at 7 days after seeding is summarized in Figure 6(c). After 7 days of incubation, the cells on both samples grow and proliferate. Comparing the two samples, the number of cells on the O2 PIII PTFE \((9.48 \pm 0.13) \times 10^4\) is larger than that on the control \((4.29 \pm 0.25) \times 10^4\). Therefore, cell adhesion is easier on the modified PTFE surface and as time elapses, the number of cells between the treated and untreated PTFE becomes more obvious.

Conclusions

After treatment by long-pulse and high-frequency PIII, the structure of the PTFE surface exhibits notable changes. The treating time also affects the contact angle and roughness of the surface. A more hydrophobic (with a water contact angle of 160°) and rougher surface is formed after a 1-hour treatment compared to half an hour.\,[19] The contact angles decrease with time and stabilize at 148° after 30 days. Although XPS shows that smaller amounts of the C−O and C=O surface functional groups are formed, the superhydrophobic surface properties appear to arise from the surface roughness. XPS depth profiles also indicate that oxygen ions are not implanted into the PTFE samples. MC3T3-E1 osteoblasts cultured on the treated and control PTFE samples indicate enhanced cell growth and proliferation on the PIII treated sample. Our results suggest that the rough surface caused by energetic ion bombardment in concert with plasma modification is the predominant factor of the enhanced cell attachment and growth. Plasma treatment using noble gases such as Ar, He, and which do not alter the chemical properties of PTFE’s surface may also lead to interesting results and more work is being conducted in our laboratory.

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