び P. Maguire 教授が研究チームを率いている。

金属ステントの DLC コーティングおよび実用化には極めて積極的で，法律的にも抗血栓性・デザイン・コスト・治具に至る細かい点まで詳細に認識していた。DLC の医療応用に関しては，特に Si 添加 DLC をメインテーマとして研究しており，我々の F-DLC と同様に良好な抗血栓性を報告している[9),10)]。Medical application がグループのメインテーマであるが，カーボンナノチューブ，ペットボトルの内側コーティング，大気圧プラズマ技術およびナノパターニングなども実施していた。このグループの特徴は，University of Nottingham よりも，他分野の研究者を 1 つのグループ内に集めていることだ。材料科学，プラズマ技術者，解析者，化学者，医師，経営学者等の集団で，意識的にそうしている。Queens University と共同で，「NanoTec」という会社を設立し，多くの企業と連携してベルファスト近郊の産業の復興に努めていた。北アイルランドはここ 10 年，経済が好調で科学技術に多額を投資しており，その一貫としてこのグループができ上がってきたようである。施設は極めて綺麗で，最新装置が広いスペースにゆったりと置いてある。国からの支援だけでなく企業との連携も多く，特にボストンサイエンティフィックやメドトロニックのような大手医療機器会社と共同開発を実施していた。日本のテルモの研究開発状況も良く知っており，「医療機器の実用化」という観点からは本施設は世界的にも群を抜いていると考えられる。

4. 7. 3 スイス連邦材料試験研究所(EMPA)：Laboratory for Nanoscale Materials Science

EMPA はチューリッヒの郊外にあり，スタッフを含め研究員総数は約 500 人という大きな国立研究所である。我々は，DLC のいくつかの総説[18)]を発表している R. Hauert 博士(Head of Surface Technology)および EMPA の施設長の L. Schlapbach 教授を訪問した。スイスというお国柄からか，時計部品(歯車)への DLC コーティングの実用化の話は意味深であった。高級時計は，オイルで摺動を制御するが，それは 20 年間の保障であるらしい。したがって 20 年くらいたつと寿命ということになる。DLC は半永久的ということで実用化されている。ただし，まだ 20 年経っていないので結果は出ていない。また時計バンドでも，金属アレルギーを防ぐ目的で DLC コーティングは実用化されている。血液循環ポンプや心臓弁への応用例も多くあるらしいが，実用化されているかは不明である。たとえばイタリアの医療機器大手のソーリン(SORIN)は DLC に関連した医療機器を数種製品化しているらしい。「開発から製品化」への議論になったが，スイスの場合 "Swiss federal office of public research" が監督して，規則は米国の FDA (Food and Drug Administration：食品医薬品局)に比べゆるいらしい。製品をワールドワイドに展開する場合は FDA の認可も取得したければならない。全体として，医療機器の種類によりケースバイケースだが，まず新開発品をヨーロッパで製品化し，様子を見ながら世界展開できるところに彼らの強みがあると感じた。

5. おわりに

全体として，今後表面改質を利用して新しい医療機器を世界に発信するに当たり，以下の点が重要と考えた。

1) 外国製品は外国人の体型・体質に即していることが多々あり，日本人に合わない場合がある(たとえば，血管径は欧米人より日本人の方が小さい場合が多く，器具を導入する際に血管径に対して器具が太すぎる・・など)。製品が日本人に適さないと判断できるのは日本の病院で患者に使用される段階になってからである。日本人のために新たな改良を欧米の日本販売代理店に陳情したとしても，本国との連絡には時間がかるばかりでなく，欧米企業は医療業界においても常に「マーケット」という考えが強くあるため，欧米のニーズを最優先し，多大な費用がかかる変更に消極的な場合も多く見受けられる(変更に数年単位かかることもありうる)。これらの事態は，患者様の利益という点においても，国民の健康を守っていく医師の立場からみても由々しき事態であり，安全保障上の理由からも純日本製の製品の開発・販売の促進が重要である。これは，現在資金力のないが技術力をもった中小企業を育成し，医療器具開発・販売メーカーの層を厚くすることが急務である。

2) 日本独自の技術(日本人の器用さ)で西欧技術を凌駕するような製品を突き詰めるべきである。たとえは，金属ステントのデザインなどにも我が国固有の西陣織のような技術が適応できる可能性がある。

3) 新規の製品開発だけを狙うのではなく，従来の日本型開発方法を模倣した修正型開発方式は適用することも重要なステップとなりうる。

以上の点を実現するためのチームとして，臨床医，工学・理学博士，国内企業，各省庁など，各分野のエキスパートが密な研究協力体性をとることが必要である。このようなチームは形式上，近年実現されつつあるが，実情は厳しく，本当の意味でも連携ができているプロジェクトは少ないといってよい。患者様のニーズを正確に把握し，実際の臨床現場で必要とされている日本国内発信型のメディカルデバイスを開発，実用応用につなげていきたいと考えている。

(2005-9-6 受理)

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Lubrication performance of diamond-like carbon and fluorinated diamond-like carbon coatings for intravascular guidewires

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Abstract

Diamond-like carbon (DLC) and fluorinated DLC (F-DLC) films were deposited on SUS316L guidewires using radio frequency (RF) plasma enhanced chemical vapor deposition (CVD), and the lubrication performance of DLC- or F-DLC-coated guidewires was then evaluated under in vitro conditions using a novel friction simulator developed for this study. Scanning electron microscopy (SEM) demonstrated that DLC or F-DLC film completely coated the specimens (SUS316L guidewires) and that polishing scars were substantially reduced. In the torturous vessel model, DLC- or F-DLC-coated guidewires exhibited significantly improved lubrication performance (by approximately 30% over that of uncoated wires). DLC and F-DLC films are thus promising candidates for lubricious coating of intravascular guidewires.

Keywords: Diamond-like carbon; Fluorinated diamond-like carbon; Lubrication; Guidewire

1. Introduction

Intravascular guidewires are widely utilized to guide and place catheters for diagnostic angiography and balloon catheters for percutaneous transluminal angioplasty (PTA) in the vascular lumens of human body. For example, to perform diagnostic angiography followed by therapeutic intervention in the human vascular system, a guidewire is first inserted into the vessel and guided through the tortuous path desired for the catheter, after which the catheter is threaded over the guidewire. As the catheter is inserted and advanced over the guidewire, it ultimately negotiates the same tortuous path. However, the inability to pass the guidewire through tight stenoses or torturous vessels is the most common cause of failed angioplasty. Thus, the lubrication performance of guidewires is critical for successful procedures.

After the catheter is placed in its final operative position, the diagnostic guidewire can be removed and the catheter is used to perform angiography with contrast material; however, guidewires for PTA and stent placement usually remain in the target vessels throughout the entire procedure. Because the duration of many of these interventional procedures is relatively long, the possibility of thrombotic embolus formation on the guidewire surface increases the risk of downstream occlusion in the coronary, cerebral, and femoropopliteal regions. Therefore, the distal part of an intravascular guidewire should be not only flexible, nonkinking, radiopaque and lubricious, but also antithrombogenic.

Hydrophilic coatings have a relatively recent advance in commercial catheter and guidewire technology. Hydrophilic catheters and guidewires facilitate difficult catheterization, particularly for therapeutic interventional procedures in which extremely distal access is required [1]. This type of coating is believed to be sufficiently lubricious for catheterization and to

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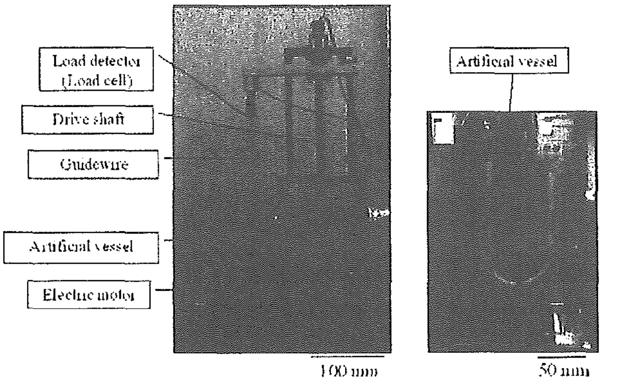

Fig. 1. Friction simulator for guidewires consisting of a fixed frame with a drive shaft linked to an electric motor, a load cell, a guidewire and an artificial vessel (PTFE; 6 mm i.d.). The artificial vessel was set in an acrylic resin tub in order to facilitate simple changes in shape.

reduce damage to the vessel wall during advancing and retraction based on the clinical experience that such coatings facilitate the penetration of stenoses. However, Leach et al. [2] reported that hydrophilic coatings were at least as thrombogenic as uncoated nonhydrophilic materials. This supports our observation of clotted blood on hydrophilic guidewires after use and our difficulties in inserting guidewires into angiographic catheters placed in the target vessel unless the guidewire is first wiped with a piece of gauze dipped in heparinized saline. On the other hand, guidewires are sometimes coated with polytetrafluoroethylene (PTFE) or silicon overlays as the hydrophobic coating. However, these coatings also have problems of thrombus formation in a clinical setting.

Diamond-like carbon (DLC) films are dense amorphous carbon films characterized by their hardness, wear resistance, electrical resistance and chemical inertness [3,4]. The mechanical properties of these films fall between those of graphite and diamond; the films have a low-friction coefficient, low wear rate, high hardness, excellent tribological properties and good corrosion resistance [5,6]. In addition, a number of promising results have indicated good blood compatibility, antithrombogenicity and biocompatibility in various cell culture [5,7–14] and animal models [11,15]. DLC coatings are therefore being considered for widespread clinical use as surface coatings for coronary stents [16–18], heart valves [19] and orthopedic implants [11] for humans. Application of DLC coatings to intravascular guidewires would therefore be rational because these coatings have advantageous tribological properties together with antithrombogenic characteristics.

Our previous study [20] confirmed a dramatic reduction in the number and activation of adhered platelets after doping conventional diamond-like carbon films with fluorine (F-DLC). In this investigation, we deposited DLC or F-DLC films on intravascular guidewires using the radio frequency (RF) plasma enhanced chemical vapor deposition (CVD) method and evaluated the lubrication performance of DLC- or F-DLC-coated guidewires under in vitro conditions using a novel friction simulator developed for this study.

2. Experimental

DLC and F-DLC films were prepared by RF plasma enhanced CVD in a parallel plate reactor with hexafluoroethane (C_2F_6) and acetylene (C_2H_2) gas. DLC films were deposited from C_2H_2 and F-DLC films were from a mixture of C_2H_2 and C_2F_6, respectively. F-DLC films were deposited under a partial pressure of C_2F_6 at 60% of the total pressure. The RF (13.56 MHz) power, total pressure and gas flow rate were fixed at 400 W, 13.3 Pa and 20 ml/min, respectively. Coatings were deposited to approximately 40 to 50 nm on SUS316L guidewires (0.4 mm i.d. $\times$250 mm). Surfaces of uncoated SUS316L guidewires, DLC-coated guidewires and F-DLC-coated guidewires were observed by scanning electron microscopy (SEM).

We designed a friction simulator in order to evaluate the lubrication behavior of guidewires in vitro. The friction simulator consists of a fixed frame with a drive shaft linked to an electric motor, a load cell, a guidewire and an artificial vessel (polytetrafluoroethylene, PTFE; 6 mm i.d.), as shown in Fig. 1. Frictional forces were measured by the load cell while moving the guidewire up and down along the inner lumen of the artificial vessel at a velocity of 10 mm/s.

In this study, we tested uncoated, F-DLC- and DLC-coated guidewires in order to evaluate lubrication performance in two types of vascular setting, as shown in Fig. 2. Because it was difficult to reproduce the clinician's hand manipulation and to avoid bending the guidewire during insertion using the drive shaft, particularly in the torturous vessel model (Fig. 2B), we collected and analyzed the maximum load (kgf) measured when the guidewire was retracted from the artificial vessel as lubrication performance data. Experiments were repeated 30 times with each guidewire in each vascular setting. The ratio of average maximum load for DLC- or F-DLC-coated guidewires to that for uncoated wires was calculated. Results are expressed as mean±standard deviation (SD). Data were compared

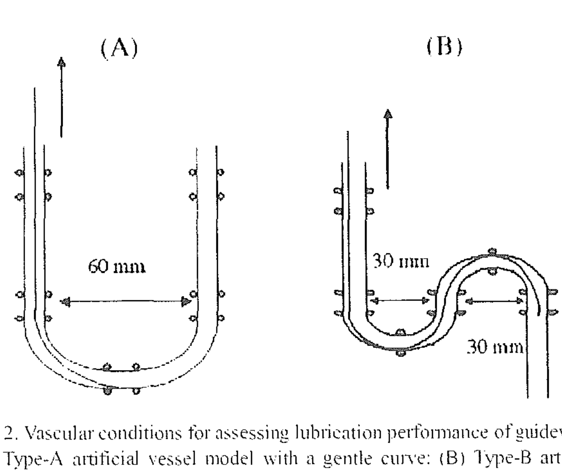

Fig. 2. Vascular conditions for assessing lubrication performance of guidewires: (A) Type-A artificial vessel model with a gentle curve; (B) Type-B artificial vessel model with a tortuous path.

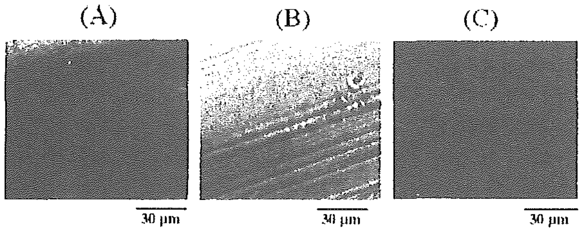

Fig. 3. SEM images of guidewire surfaces. (A) SUS316L, (B) DLC-coated, and (C) F-DLC-coated.

statistically by paired t-test and $P<0.05$ was considered to be statistically significant.

3. Results and discussion

Fig. 3A is an SEM image showing the polishing scars on the surface of the SUS316L guidewire. The DLC and F-DLC films completely covered the specimens (SUS316L guidewires) and substantially reduced the appearance of polishing scars (Fig. 3B, C).

The average maximum load for DLC- or F-DLC-coated guidewires was compared to that of uncoated SUS316L guidewires under type-A (Fig. 2A) and type-B (Fig. 2B) vascular conditions. Fig. 4A and B show the lubrication performance data for DLC- or F-DLC-coated guidewires versus uncoated wires. Under type-A conditions, the lubricity of the DLC- or F-DLC-coated guidewires was almost the same as that of uncoated guidewires. There were no statistically significant differences between the DLC-coated, F-DLC-coated and uncoated groups. On the other hand, under type-B conditions, in which the vessel was more torturous (Fig. 2B), the lubricity of the DLC- or F-DLC-coated guidewires was improved by approximately 30% over that of uncoated wires ($P<0.05$). However, there was no statistically significant difference between the DLC and F-DLC groups. The friction drag against the inner wall of the artificial vessel under type-B conditions was thought to be higher than under type-A conditions, and thus the efficacy of DLC and F-DLC coatings was apparent in the more tortuous vessel model. This suggests that DLC- or F-DLC-coated guidewires would be able to more easily navigate tortuous paths and pass through difficult regions within arteries in clinical settings. In contrast, the type-A artificial vessel has a relatively gentle curve with a large curvature, and the friction drag against its inner wall was thought to be low; therefore, lubrication performance was not enhanced by coating with DLC or F-DLC. In clinical settings, advancing a guidewire through arteries with gentle curves is relatively simple, irrespective of the guidewire used. Therefore, performance in tortuous vessels is more important for evaluating the lubricity of guidewires.

In lubricity tests, Maguire et al. [21] and McLaughlin et al. [22] found that the friction coefficient for conventional DLC coatings is better than that for PTFE coatings for guidewires. Finished catheters and guidewires are typically spray coated with a thin layer of PTFE, silicone overlays and other hydrophilic coatings, which reduce the friction coefficient. However, spraying does not produce coatings that are sufficiently smooth and uniform. Consistency is prized by designers whose task can be complicated by core material of varying diameter. Lubricious coatings have been used in a wide variety of medical devices, including those for use in cardiology, urology and neurology, and for many diagnostic applications. Angioplasty balloons, Foley catheters, urethral stents, microcatheters and guidewires all benefit from incorporation of lubricious coatings. Hemodialysis equipment is also coated to reduce patient trauma.

Our coating thickness is 40 to 50 nm in thickness, —much thinner than a conventional spray coating, which usually ranges from approximately 0.001 to 0.01 inches [in] (approximately 2.5×10^{4} to 2.5×10^{5} nm) [22]. This super-thin coating lets designers increase the diameter of the core material. For example, device specifications normally limit guidewire cores used in percutaneous transluminal coronary angioplasty (PTCA) to a diameter of 0.014 in. To allow for the coating thickness, the specifications usually call for an uncoated wire diameter of 0.013 in. Coatings as thin as 40 to 50 nm can be achieved with minimal dimensional changes to the substrate. Furthermore, the coating thickness can be customized to meet manufacturers' specifications. Thus, designers can increase the size of the material to add stiffness when using DLC or F-DLC coatings, which is a key property of the PTCA guidewire core.

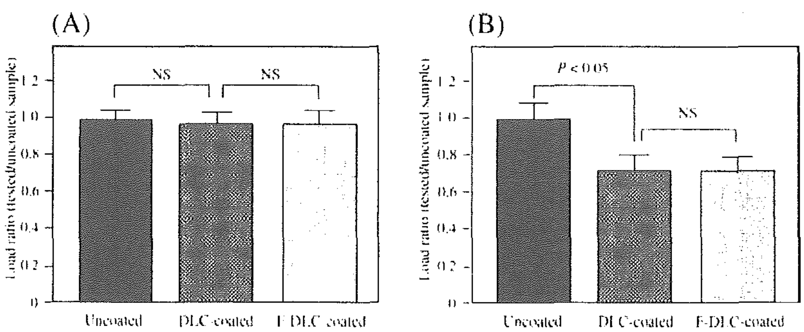

Fig. 4. Lubrication performance data for SUS316L, DLC-coated and F-DLC coated guidewires in under type-A (A) or type-B (B) vascular conditions (see Fig. 2). Maximum load ratio (DLC or F-DLC-coated wires/uncoated guidewires) was calculated ($n=30$ times/each group). Values are expressed as mean±standard deviation (SD) and were compared statistically by paired t-test, with $P<0.05$ being considered statistically significant.

We recently reported the results of a quantitative and morphological study on platelet adhesion to DLC films, F-DLC-coated silicon (Si) and bare Si substrates incubated in platelet-rich plasma [20]. In that study, it was found that F-DLC possessed significantly better antithrombogenicity when compared with the other tested samples. In addition, F-DLC coating not only reduced the number of adherent platelets, but also inhibited platelet activation on the film. Another study reported that surface fluorination can be used to create surfaces with improved blood compatibility, hydrophobicity and chemical stability [23]. Incorporation of fluorine into DLC films is reported to greatly reduce film hardness while largely preserving other beneficial properties [24]. Therefore, the antithrombogenic and elastic features of F-DLC would be advantageous for coating three-dimensional medical devices when compared with conventional DLC coatings. Magurie et al. [21] recently reported that doping with silicon and the use of an a-Si:H interlayer in guidewire coating help minimize the risks of adhesion failure and film cracking. In the present study, we demonstrated the superior lubricity of DLC and F-DLC coatings when compared with uncoated stainless steel; however, further study is needed in order to investigate the durability and adhesion strength of DLC- or F-DLC-coated guidewires using an a-Si:H/a-Si:C:H interlayer.

Other groups are working on a variety of blood-compatible coatings, including a photoheparin formulation. Heparin coating has also showed a reduction in platelet attachment; however, it is unstable in the body and is derived from animal cells. Regulators in Europe, United States and Japan are concerned about the possible side effects of heparin, which could make it difficult to bring heparin-coated products to market. DLC and F-DLC coatings would thus be possible nonbiologic alternatives to heparin.

4. Conclusions

DLC or F-DLC films were deposited on SUS316L guidewires using the RF-CVD method and the lubrication performance was evaluated and compared with uncoated guidewires under in vitro conditions. The results obtained were as follows:

(1) DLC- or F-DLC-coated SUS316L guidewires were observed by SEM, showing that DLC and F-DLC films were deposited evenly over the entire specimen, reducing the appearance of polishing scars.
(2) DLC and F-DLC coatings improved lubricity of coated guidewires by approximately 30% when compared with uncoated guidewires (SUS316L) under in vitro conditions with strong friction drag.
(3) Further study is needed to investigate coating strength and durability for clinical use.

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Antithrombogenicity of fluorinated diamond-like carbon films

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Abstract

Effects of doping with fluorine to diamond-like carbon (DLC) films on the antithrombogenicity were investigated by changing its content. Fluorinated DLC (F-DLC) films were prepared on silicon (Si) substrates using radio frequency (RF) plasma enhanced chemical vapour deposition (CVD) by changing the ratio of hexafluoroethane (C_2F_6) and acetylene (C_2H_2). The contact angle measurements of human whole blood droplet on Si, DLC and F-DLC were 24.2°, 60.8° and 95.3°, respectively. Furthermore, the static evaluation of F-DLC films incubated with platelet-rich plasma (PRP) showed dramatic reduction of platelet adhesion and activation on the surface. It was found that the addition of fluorine into DLC films much improved antithrombogenicity, which was clearly shown by scanning electron microscopy (SEM) with statistical analysis. F-DLC coating can be a great candidate for developing antithrombogenic surfaces in blood contacting materials.

Keywords: Fluorinated diamond-like carbon; Hydrophobicity; Platelet adhesion; Antithrombogenicity

1. Introduction

Biomaterial implants such as vascular grafts, artificial heart valves or interventional devices (stents, guidewires and catheters) have been gaining widespread use with development of medical engineering. Thrombogenic complication remains as one of the main problems for blood contacting implants, which triggers the life-threatening device failure. For example, it has been reported that the restenosis after coronary stenting statistically occurs 20% to 40% [1–4]. Recent study has shown that the thrombus formation after intraarterial stent implantation provides a stimulus for neointimal hyperplasia and, if excessive, can result in stent thrombosis at sites of coronary stenting in humans [4]. Surface modification techniques are indispensable for improving both the mechanical and physical properties of these implants in direct contact with the blood and tissue.

Recently, diamond-like carbon (DLC) films have received much attention because of their antithrombogenicity, which inhibits platelet adhesion and activation [5–7]. However, the blood coagulation mechanism on DLC films in biological environment has not been well understood so far. There have been several reports that cell adhesion on DLC films is related to surface energy and wettability [8–12]. They suggested that hydrophobic surface tends to inhibit blood cell adsorption. As for hydrophobic property, it is well known that fluorocarbon polymers present great water-shedding characteristics. Thus, we consider that the addition of fluorinate has potential to develop haemocompatibility of materials.

2. Experimental

2.1. Film preparations and characterization

Conventional DLC and fluorinated DLC (F-DLC) films were prepared on Silicon (Si) substrates using radio

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frequency (RF) plasma enhanced chemical vapour deposition (CVD) method by changing the ratio of hexafluoroethane (C_2F_6) and acetylene (C_2H_2). The RF (13.56 MHz) power and total pressure were fixed at 200 W and 13.3 Pa, respectively. DLC films were deposited from C_2H_2 and F-DLC films from a mixture of C_2H_2 and C_2F_6, and the thickness of DLC and F-DLC films was 40 to 50 nm, respectively. According to partial pressure of C_2F_6, each F-DLC film was denoted as follows: for example, F-DLC20 indicates that F-DLC films were deposited under partial pressure of C_2F_6 at 20% of the total pressure.

Surface chemical compositions and bonding states of DLC and F-DLC films were measured by the X-ray photoelectron spectroscopy (XPS; JPS-9000MX, JEOL). The wettability of Si, DLC and F-DLC80 were evaluated by measuring the static contact angles between a droplet of human whole blood (10 μl) and the samples surfaces.

2.2. Platelet adhesion and activation

Human whole blood (45 ml) from a healthy volunteer without any medication for at least 10 days was collected and mixed with 5 ml of acid-citrate-dextrose (ACD), and then the blood was centrifuged at 180 ×g for 10 min to separate the blood corpuscles, and the resulting platelet-rich plasma (PRP) was prepared. Subsequently, the rest of whole blood was centrifuged at 2000 ×g for 20 min to obtain the platelet-poor plasma (PPP). The density of platelets in PRP was adjusted to a concentration of 2×10^5 cells/μl by diluting with PPP. After rinsing samples with phosphate-buffered saline (PBS), the samples disks with a surface area of 100 mm^2 were incubated in a 24-wells plate with 2 ml of adjusted PRP for 60 min with 5% of CO_2 gas at 37 °C (n=5 disks for each sample). Thereafter, the supernatant was discarded, and the samples were washed with PBS. The adherent platelets were then fixed with 1 ml of freshly prepared 1.0% of glutardialdehyde for 60 min at room temperature. After fixation, the samples were washed and dehydrated in a graded ethanol series (20%, 40%, 60%, 80%, 100% and 100% for 15 min each), as described previously [13]. Dehydrated materials were put in a vacuum chamber and dried overnight. The entirely dried materials were coated with gold and investigated by a scanning electron microscopy (SEM; S-3100H, HITACHI).

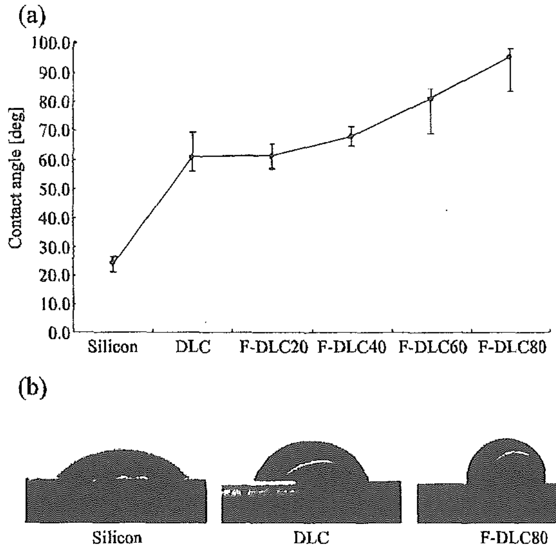

Fig. 2. Contact angle measurements of 10-μl whole human blood droplet on the sample surface (wettability). The values of contact angle dramatically increased with increasing the ratio of doped fluorine (a). F-DLC80 showed the most hydrophobic property (b).

Adhering platelets were manually counted on photographs per unit area (6000 μm^2). Results of the experiments are expressed as means of counts/unit area and standard error (SE). Values were compared statistically by unpaired t test. Results with $P<0.05$ were considered to be statistically significant. Additionally, the morphological shape changes were categorized to Goodman et al. and

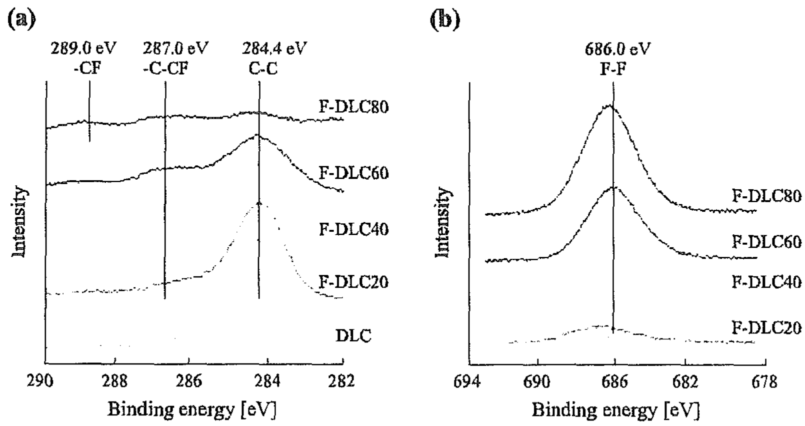

Fig. 1. XPS spectra of DLC, F-DLC20, F-DLC40, F-DLC60 and F-DLC80. (a) C1s XPS. (b) F1s XPS.

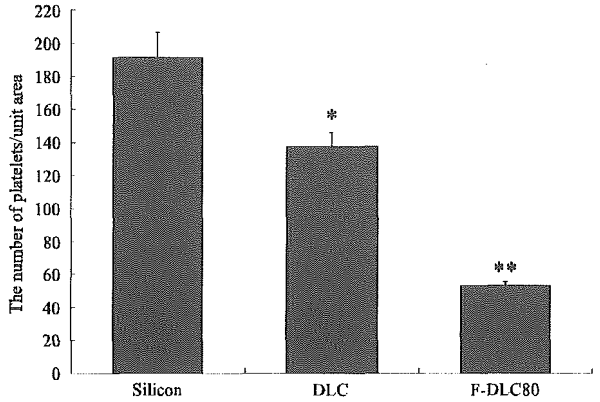

Fig. 3. The number of adhering platelets counted on Si substrate, DLC and F-DLC80. The number of platelets per unit area (6000 μm^2) was counted. The values are the mean of five areas of each sample, while the error bars denote the standard deviation. * denotes significant difference from control Si substrate (*P<0.05) .** denotes significant difference from Si or DLC (**P<0.001).

Allen et al. [14,15] as (I) round or discoid, (II) dendritic or early pseudopodial, (III) spread-dendritic or intermediate pseudopodial, (IV) spreading or late pseudopodial and (V) fully spread.

3. Results and discussion

3.1. Chemical compositions and bonding

Fig. 1(a) and (b) shows the local spectra of C1s and F1s for DLC, F-DLC20, F-DLC40, F-DLC60 and F-DLC80 by XPS. The horizontal axis corresponds to binding energy [eV] and the vertical axis to intensity. By taking curve fitting to each spectrum, they were well decomposed into main three peaks, which centered at ~284.4, ~287.0 and ~289.0 eV, respectively. Due to the surface contamination and charging effect during XPS analysis, it is very complex and also still controversial to identify these peaks.

As shown in Fig. 1, the peak intensity for C–C bond (284.4eV) gradually decreased (Fig. 1(a)) and the peak intensity for F–F bond (686.0 eV) gradually increased (Fig. 1(b)) with increasing pressure of C_2F_6 gas during deposition. This indicates that the pressure of C_2F_6 influences the ratio of fluorine on the topmost surface of films. Furthermore, in the spectra of F-DLC films, there were some peaks indicating bonds of carbon and fluorine. The spectra of F-DLC20, F-DLC40 and F-DLC60 showed the existence of C–CF bond (287.0 eV) as well as C–C bond. The spectrum of F-DLC80 showed the existence of C–F bond (289.0 eV) in addition to C–CF and C–C bonds. Bonding structures of carbon and fluorine show low polarizability, which leads to low surface energy with increasing the hydrophobicity of F-DLC films.

3.2. Contact angle measurements

Fig. 2 shows the results of contact angle measurements for three different samples. These results indicated the dramatic improvement of wettability for DLC and F-DLC films. The contact angles of human blood on Si, DLC and F-DLC80 were 24.2°, 60.8° and 95.3°, respectively. For the DLC films, the contact angle increased by 2.51 times compared to that of Si substrate. In particular, F-DLC80 is the most hydrophobic, and the contact angle for F-DLC80 increased by 3.94 times compared to that of Si.

The more hydrophobic a surface is, the higher the interfacial free energy between the solid and liquid phases. The interfacial free energy determines the wetting characteristics and hence the wall shear stress generated when the liquid comes into contact with the surface. It is considered that the polarization of C–F and C–CF bonds on the topmost surface of F-DLC80, proven by the XPS analysis, can lower the surface energy and the results in the increase in contact angles.

3.3. Platelet adhesion and activation

Platelets play a pivotal role in thrombogenicity of biomaterials in blood contacting applications, and the reduction of platelet adhesion and activation is determinant for the eventual success of any application. The initial local response to foreign surface in the body is mainly catalyzed by surface-absorbed proteins, which trigger numerous processes such as cellular activation, inflammatory and complement activation and attraction of circulating platelets.

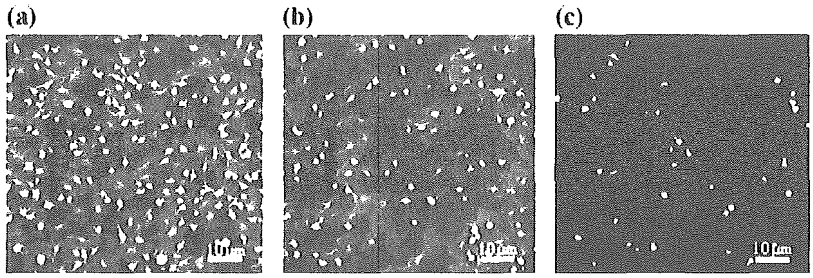

Fig. 4. Morphology of adherent platelets on (a) Si, (b) DLC and (c) F-DLC sufaces (60 min incubation in PRP) observed using SEM. Si substrate: dense platelet layers (categories IV and V). DLC: lower density of platelets compared to Si (categories III to V). F-DLC80: a few platelets (categories uniformly I to V).

During activation, the platelets attach to the sample surface, and they change in shape in developing pseudopodia versus their activation level [12]. Thus, the investigation of platelet adhesion must support the thrombogenicity evaluation of biomaterials.

Fig. 3 shows the adherent platelets counts on the three different samples by SEM. This result clearly demonstrated that the number of platelets per unit area for DLC or F-DLC80 was significantly smaller compared to that of Si substrate ($P<0.05$ and $P<0.001$, respectively). In addition, the number of platelets on F-DLC80 was significantly smaller than that of DLC ($P<0.001$). The morphology of the attached platelets after 60 min of incubation is displayed as shown in Fig. 4. Si substrate showed a dense platelet layer with predominantly spread platelets (categories IV and V) (Fig. 4(a)), whereas, on F-DLC80, a few platelets adhered, and the categories of platelets uniformly varied between I to V (Fig. 4(c)). DLC films showed that almost all the platelets were categorized into III to V (Fig. 4(b)).

In this present study, DLC and F-DLC coatings could suppress the adhesion and activation of platelets compared to Si substrate. The surface that promoted the greatest spreading of platelets, i.e., the Si substrate, is the most hydrophilic in the tested samples. In contrast, F-DLC coating, which caused less activation, was most hydrophobic in the tested samples. This suggests that the wettability of a biomaterial surface determines in part its blood compatibility. However, it has been described to be influential that blood compatibility is not solely determined by wettability and also the specific chemical composition, interfacial free energy and a higher ratio of albumin/fibrinogen adsorption of biomaterial surfaces [16,17]. The mechanism of biomaterial-associated thrombosis is not fully clear. It is very complicated to determine all factors that contributed to novel antithrombogenic properties of F-DLC films in this study. Further in vitro and in vivo studies are needed to investigate all of the factors related with biomaterial-associated thrombosis.

4. Conclusion

We have presented an engineering analysis of fluorine-doped DLC films and quantitative and morphological studies on platelet adhesion to DLC films or F-DLC coated Si and bare Si substrate incubated in PRP. In this study, we described a novel antithrombogenic effect by doping with fluorine into DLC films compared to Si substrate and conventional DLC. Our experiments showed that an addition of fluorine into DLC films enhanced the water-shedding properties. The number of platelets per unit area decreased in order of Si, DLC and F-DLC80. It was found that F-DLC showed the best antithrombogenicity among the tested samples. In addition, DLC and F-DLC coating inhibited the platelets activation, as well as the number of platelets on the film surface.

The presented F-DLC appears to be a promising candidate coating material for blood contacting devices, such as interventional devices, artificial organs and pacemakers. However, more basic study and long-term implantation are needed.

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