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Topographic features of extracorporeal circulation circuit surface observed by atomic force microscopy; coated or non-coated?

Atomik Kuvvet mikroskopisi ile incelenen ekstrakorporoyal dolaşım devrelerinin yüzeylerinin topografik özellikleri; kaplı ya da kapsız ?

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Öz

Amaç: Kalp cerrahisi sırasında kullanılan kalp akciğer makinasında bulunan ekstrakorporyal dolaşım (EKD) devrelerinin polimer yapılı parçalarının doku ya da özellikle kan gibi biyolojik sıvılarla teması önlenemez enflamatuvar ve pıhtılaşma reaksiyonlarına sebep olur. Bu reaksiyonların sebepleri arasında kullanılan malzemenin kimyasal özellikleri yanında temas yüzeyinin fiziksel özellikleri de önemlidir. Atomik kuvvet mikroskobu (AFM) ince bir borunun yüzey morfolojisini analiz etmek ve yüksek hassasiyetle kalınlığı, pürüzlülüğü ve yüzeyin eğiklik ve basıklık gibi asimetrik değerleri hakkında bilgi almak için kullanışlı bir yöntemdir. Bu çalışmanın amacı EKD devrelerinde enflamasyon ve pıhtılaşma gibi reaksiyonlarının ana sebeplerinden birisi olduğu düşünülen topografik özelliklerin AFM ile ortaya konmasıdır. Yöntemler: Bu amaçla kaplamasız (n=3), heparin (n=3) ve fosforil kolin (n=3) kaplamalı EKD devreleri ameliyat öncesi ve ameliyat sonrası AFM ile görüntülenmiştir ve yüzey fotoğrafları, 3d topografisi, kesit görüntüleri ve yüzeylerinin eğiklik ve basıklık özellikleri değerlendirilmiştir. Bulgular: Atomic kuvvet mikroskopisi görüntüleri bu devrelerden hiçbirinin yüzey özelliklerinin biyo-materyal için ideal biyouyumlulukta olmadığını göstermiştir. Sonuç: Biyo-materyal yapımında kullanılan malzemelerin kimyasal yapılarının biyo-uyumluluk için yüksek öneme sahip olduğu kadar biyomalzeme yüzeylerinin mikro / nano ölçekli topografik özelliklerinin geliştirilmesi biyo-uyumu arttırmak için potansiyel vaat eden önemli bir stratejidir.

Anahtar Kelimeler: Atomik kuvvet mikroskopisi; Ekstrakorporal sirkülasyon devreleri; basıklık; Polimer biyomalzeme; eğiklik

Abstract

Objective: Polymer based materials used in the manufacture of extracorporeal circulation (ECC) circuit that is the main part of the heart lung machine used in cardiac surgery, poses the unavoidable inflammatory and coagulation reactions because of the contact with the tissue and biological fluids particularly the blood. The reason for these reactions may include chemical properties of the material used but the surface properties of the contact surface are also important. Atomic force microscopy (AFM) is useful method to analyze the surface morphology of a thin line and obtain information about the thickness, roughness and height asymmetries values such as the skewness and kurtosis of the line. The aim of the study is demonstrating the ECC circuits' topographic features that hypothesized as one of the main reason for side effect reactions as inflammation and coagulation.

Keywords: Atomic force microscopy; extracorporeal circulation circuits; kurtosis; Polymer biomaterial; Skewness

Introduction

The hemocompatibility of the polymer biomaterials, that has to be used in medical applications which have intense and prolonged direct contact with blood, is expected to be high (1). Adhesion of proteins on the foreign surfaces after contact with blood activates a variety of cells including mainly the platelets and immune cells, complement, kinin and immune system and the coagulation cascade, and poses the side effect reactions (1-3). The severity of these reactions varies according to inner surface chemical characteristics and topographical structure of the polyvinyl chloride (PVC) lines and their contents used in the other parts of the circuit. That's why constituted of the main theme of development projects in the search for these types of highly biocompatibility materials or the modification of surfaces in contact with blood products in the manufacture (3). Recently highlighted limitations and problems about using polymers such as PVC in the medical field, has increased the research and development of new coating materials (heparin, phosphoryl choline, etc.) towards by improving the biocompatibility of the work. Many studies have been concerned with the effects of depth and width of grooves on surface orientation but it appears that no consensus was found on the minimal depth and width needed for biocompatibility of materials (2). However, the relation between ordered nano-topography and cell behavior is, to a large extent, unknown so far. Since materials interact with environment through their interfaces, both the kind and the strength of such interactions are largely dependent on the surface properties such as chemistry and topography of the materials (2). These surface properties may influence protein adsorption, cell interaction, host response and ultimately the mechanical damage to shaped blood cells (1,2).

The aim of the study was to investigate the coated extracorporeal circulation (ECC) circuit surface by the aspect of cell size and to emphasize the importance of the roughness of the circuit surfaces.

Methods

In this study the main purpose was demonstrating the ECC circuits' topographic features. For that purpose, heparin (n=3), phosphoryl choline (n=3) and non-coated (n=3) ECC lines' surface were analyzed with an Atomic force microscopy (AFM). The ECC line samples were investigated before cardiopulmonary bypass (CPB) and after weaning from the CPB. All the samples were washed with phosphate-buffered saline (PBS, pH= 7.4) to remove non-adherent cells (mostly erythrocytes). Subsequently the adherent cells were fixed with glutaraldehyde (2% in PBS) for one hour, rinsed with PBS, and dehydrated with a graded ethanol series. Samples were critical point dried (CPD 030, BAL-TEC, Schalksmuhle, Germany), gold coated with a sputter coater (SCD 050, BAL-TEC, Schalksmuhle, Germany), and examined by means of a scanning- Atomic Force Microscopy (Nanomagnetics Instruments, Oxford, UK). 30x30 μm scans were obtained on each sample on randomly selected locations using tapping mode at ambient conditions.

Atomic force microscopy (AFM) is useful method to both analyze the surface morphology of a thin line and obtain information about the thickness, roughness and height asymmetries values such as the skewness and kurtosis of the line. Further, morphological features of ECC lines prepared are examined using AFM. Topographic and phase images are obtained in the non-contact mode under room conditions with typical rate of about 1 line per second with a resonance frequency of about 300 kHz. Measurements are performed with 512 scan lines. Several regions on the specimen surface are scanned to observe the similar images. Atomic force microscopy image analysis is carried out with special software written by Nanomagnetic Instrument group. Analysis is performed in the non-contact mode.

Results

Atomic force microscopy (AFM) images of non-coated (n=3) (Fig. 1a and 1b), heparin coated (n=3) (Fig 1c and 1d) and phosphorylcholine coated ECC lines (n=3) (Fig. 1e and f) before cardiopulmonary bypass (CPB) were shown in Fig. 1. Three direction (3D) topographic images of the lines which represent the roughness are very noticeable. The pre-CPB non-coated ECC line surface images revealed an average 438.18±50.25 nm roughness and the

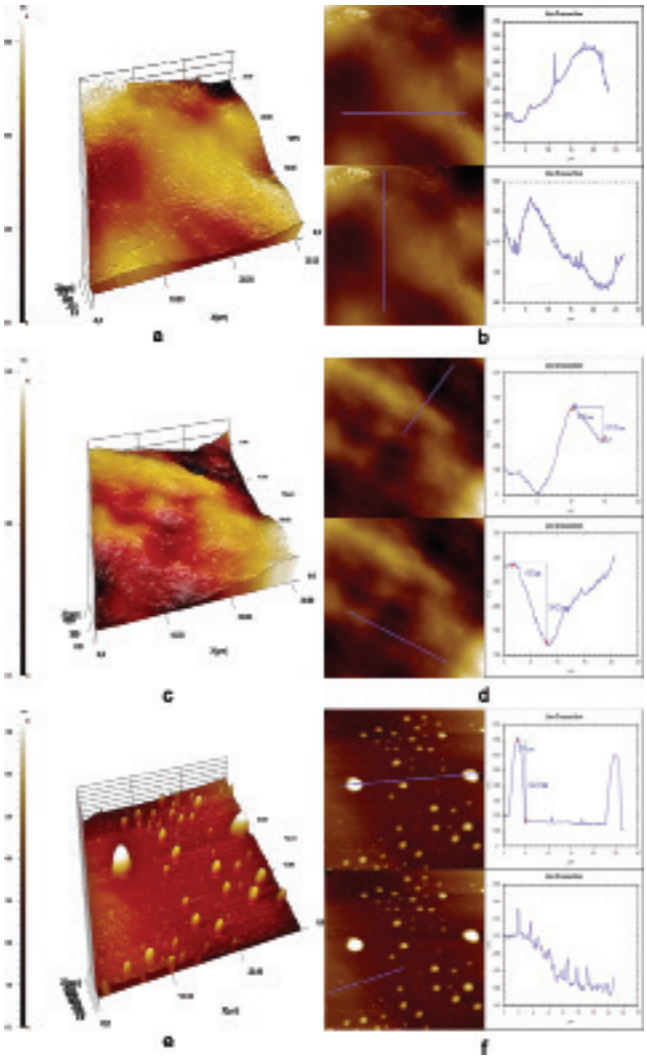

Fig. 1 Atomic force microscopy (AFM) images of the non-coated Extracorporeal Circulation Circuit (ECC) lines (n=3) (Fig. 1a and 1b), Heparin coated ECC lines (n=3) (Fig 1c and 1d) and Phosphorylcholine coated ECC lines (n=3) (Fig. 1e and f) before cardiopulmonary bypass (CPB). Three direction (3D) topographic images of the lines which represent the roughness are very noticeable. The white color represents the vertex of the height and the dark color represents the base of the height on images. Fig. 1a: The pre-CPB non-coated ECC line surface image reveals that it is too rough (Average 438.18±50.25 nm) and the height asymmetries values such as the skewness and kurtosis of the line are 1114.86±123.47 and 1317.27±220.89, respectively. Fig. 1b: The topographic images of the non-coated ECC lines (left) and cross-sections of the blue lines on the topographic images represented on the graphic (right). Fig. 1c: The AFM images of the Heparin coated ECC lines represent the relatively smooth and well-balanced surface which the average height level was 230.99±21.78 nm, the skewness was 1252.87±126.45 and the kurtosis was 1725.98±352.65. The three direction (3D) topographic surface images of the unused heparin coated ECC lines represent the bumpy surface and the rough on the surface. The black points represent the holes which may not be coated with heparin equally on the surface and the white points that are spreaded diffusely to the surface, indicate the heights which can be seen on 3D images. Fig. 1d: The topographic surface images of the heparin coated ECC lines (left) and cross-sections of the blue lines on the topographic images represented on the graphic (right) and bumpy surface has been detected on all the images. Fig. 1e: The AFM images of the Phosphorylcholin coated ECC lines before CPB. The images show roughness and adhesion on the surface of the material. It can be seen dot-like protrusion pattern (white dots) on the surface of the phosphoryl choline coated ECC line. The biggest white dot represents the highest protrusion (Fig 1e). Although the phosphorylcholine coated ECC line has a smooth ground, there are lots of irregular and different sized heights all over the surface. Fig. 1f: The average surface roughness of the phosphoryl choline coated ECC was 177.97±32.94 nm, the skewness was 1232.32±51.42 and the kurtosis was 1907.51±259.54

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Atomic force microscopy (AFM) images of the non-coated (n=3), heparin coated ECC (n=3) and phosphorylcholine coated ECC lines (n=3) after

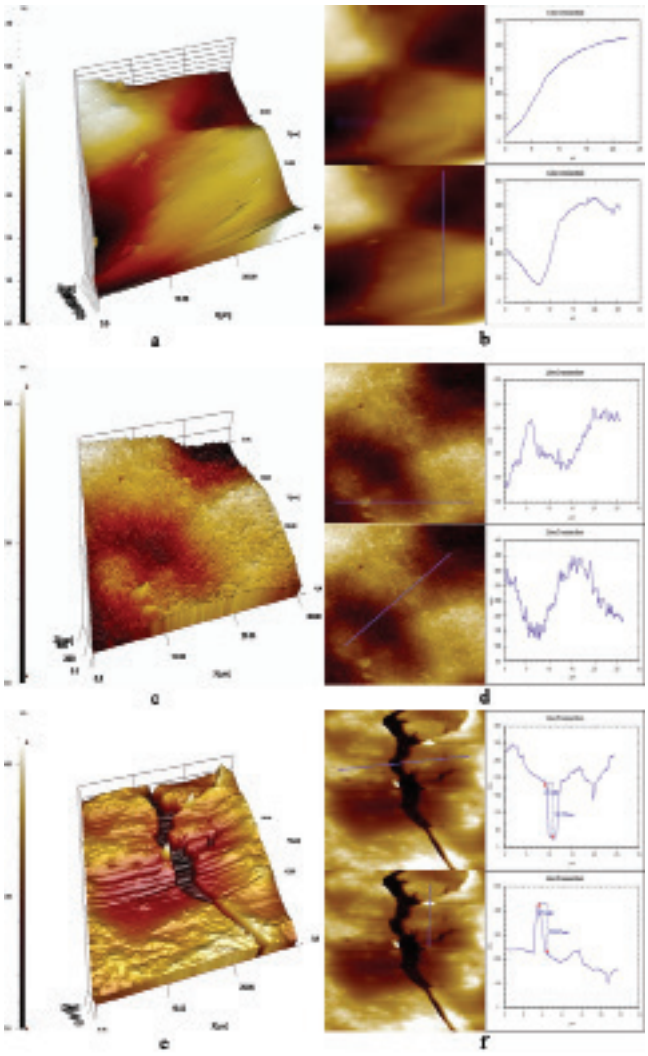

Fig. 2 Atomic force microscopy (AFM) images of the non-coated Extracorporeal Circulation Circuit (ECC) lines (n=3), Heparin coated ECC (n=3) and phosphorylcholine coated ECC (n=3) after cardiopulmonary bypass (CPB). The white color represents the vertex of the height and the dark color represents the base of the height on images. Fig. 2a: Three direction (3D) images show roughness and adhesion on the surface of the materials. The surface image of the line that used on CPB reveals a decrease on roughness (average 325.16.±45.36 nm) that may be considered as the protein and fibrin adherence. But the skewness was 1214.26±112.14 and the kurtosis was 1588.66±256.85 and thought that the surface was already rough as initial. Fig. 2b: The topographic images of the non-coated ECC lines (left) and cross-sections of the blue lines on the topographic images represented on the graphic (right) which represent the roughness are very noticeable. Fig. 2c: The 3D AFM images of the Heparin coated ECC lines after CPB. The entire surface is coated with the protein and the fibrin (inferior corner of the image) layer and the protein layer may be fragmented because of the sample preparation technique. Adhered protein and fibrin layer may be flattened the surface according the initial line surface during the CPB. Fig. 2d: The topographic surface images of the heparin coated ECC lines (left) and cross-sections of the blue lines on the topographic images represented on the graphic (right) and bumpy surface has been detected on all the images. The 3D topographic images indicate the protein and fibrin adhered surface obviously and although roughness seems to be increased (230.99±15.40 vs 334.63±23.12), skewness (1252.87±190.85 vs 1177.74±89.20) and kurtosis (1725.98±230.03 vs 1472.69±209.90) decreased because of adhered substance. Fig. 2e: The 3D topographic AFM images obviously indicate the protrusions and protein adhered surface with fissure of the phosphorylcholine coated ECC lines after CPB. The used phosphoryl choline coated ECC line' surface is coated with the protein or the fibrin layer. It has been shown that deep fissures have been occurred all over the line' surface and may be covered with the protein or fibrin layer, lose its heights lower than 200 nm and perhaps because of the thick protein/fibrin layer, it has been result some fissures which measured as 160 nm depth on the images (Fig 2e and 2f). Fig. 2f: The average surface roughness of the used phosphoryl choline coated ECC surface was 210.78±23.63 nm, the skewness was 1124.90±102.95 and the kurtosis was 1325.49±149.50

cardiopulmonary bypass (CPB) were shown in Fig. 2. The 3D images revealed the roughness and adhesions on the surface of the materials (Fig. 2a). The surface image of the line that used on CPB reveals a decrease on roughness (average 325.16.±45.36 nm) that may be considered as the protein and fibrin adherence. But the skewness was 1214.26±112.14 and the kurtosis was 1588.66±256.85 and thought that the surface was already rough as initial. The 3D AFM images of the Heparin coated ECC lines after CPB represented that the entire surface is coated with the protein and the fibrin (inferior corner of the image) layer and the protein layer may be fragmented because of the sample preparation technique (Fig. 2c). The 3D topographic images indicated that the protein and fibrin was adhered surface obviously and although the roughness (230.99±15.40 vs 334.63±23.12) was increased, the skewness (1252.87±190.85 vs 1177.74±89.20) and the kurtosis (1725.98±230.03 vs 1472.69±209.90) were decreased because of the adhered substance (Fig. 2d). The 3D topographic AFM images obviously indicate the protrusions and protein adhered surface with fissure of the phosphorylcholine coated ECC lines after CPB (Fig. 2e). The used

phosphoryl choline coated ECC line' surface is coated with the protein or the fibrin layer. It has been shown that deep fissures have been occurred all over the line' surface and covered with the protein or fibrin layer, lose its heights lower than 200 nm because of the thick protein/fibrin layer (Fig 2e and 2f). The average surface roughness of the used phosphoryl choline coated ECC surface was 210.78±23.63 nm, the skewness was 1124.90±102.95 and the kurtosis was 1325.49±149.50 (Fig. 2f).

Discussion

The biocompatibility must be considered as mechanically in three way; surface roughness, the size of biological elements interacting with surfaces, and the adsorbed proteins (2). Surface roughness can be divided roughly into three groups: (a) >2nm, (b) <2nm and >50nm, (c) <50 nm. In the range of (a), increase of roughness will simply result in more contact area for platelet surface adhesion, leading to a more thrombogenic surface; in the range of (b), particular surface topographies, such as pillars and grooves, may reduce the contact area for platelets, which can only adhere on the top of the topographic features; platelet adhesion and thrombus formation may thus be reduced; in the range of (c), the surface structures are even smaller than the pseudopods of platelets and can be considered smooth for platelets (3). Another important parameter is the size of biological elements interacting with surfaces. Large cells like monocytes (~10 μm) will react differently to the same surface topography than smaller cells like platelets (~2 μm) (4). The effective features were in the size range of platelets or below. Minelli et al indicated that increasing the feature size of the surface structures from 27nm to 1240nm encouraged von Willebrand factor adsorption, leading to platelet adhesion and consequent thrombus formation (4). Additionally, cell adhesion is related to adsorbed proteins. Considering the size scale of proteins, the protein adsorption will surely be influenced by the surface morphology considered at the nanometer scale (4).

Study Limitations

The low number of ECC line samples enrolled in the study as well as a lack of measurement of inflammatory, coagulation and compatibility related parameters are the major limitation of our study.

Conclusion

Considering the biomaterial design approaches to date, although the influence of the chemistry of the substrate is certainly of high importance, micro/nanoscale topography on biomaterial surfaces is a promising new strategy and shows potential for improving blood compatibility; for example, it could be considered to be super-hydrophobic and "super cell- phobic" state.

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